FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Microbiological Risk Assessment of Raw Milk Cheese

Risk Assessment
Microbiology Section

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ABBREVIATIONS

ANZFA	Australia New Zealand Food Authority
a_w	Water activity
CAC	Codex Alimentarius Commission
CFIA	Canadian Food Inspection Agency
cfu	Colony forming units
Codex	Codex Alimentarius Commission
CFR	Code of Federal Regulations
CP	Coagulase Positive
DAFF	Department of Agriculture, Fisheries and Forestry
DOC	Denominazione d'origine controllata (Protected Denomination of Origin)
EHEC	Enterohaemorrhagic *Escherichia coli*
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
g, ng, μg, mg, kg	Gram, nanogram, microgram, milligram, kilogram
ICMSF	International Commission on Microbiological Specifications for Foods
IDF	International Dairy Federation
IFST	Institute of Food Science and Technology
IRA	Import Risk Analysis
LP	Lactoperoxidase
MPD	Maximum Population Density
NaCl	Sodium chloride
NEPPS	National Enteric Pathogen Surveillance System
NNS	National Nutrition Survey
PCR	Polymerase Chain Reaction
s.d.	Standard deviation
STEC	Shiga toxin-producing *E. coli*
The Code	Australia New Zealand Food Standards Code
The Profile	A Risk Profile of Dairy Products in Australia
UHT	Ultra High Temperature
VTEC	Verocytotoxin-producing *E. coli*
WHO	World Health Organization

1. Executive summary

The risk assessment brings together information on the public health risks associated with the consumption of raw milk cheeses. Included in the assessment is an evaluation of the impact of cheesemaking steps on the microbiological safety of these cheeses.

The risk assessment was undertaken to answer the following questions:

(1) What are the risks to public health and safety posed by the consumption, in Australia, of raw milk cheese?

(2) What are the factors that would have the greatest impact on public health and safety along the production chain for raw milk cheese?

In order to assess the public health and safety of raw milk cheese, the scope of the risk assessment was to evaluate very hard (<36% moisture), hard (37 - 42% moisture),semi-soft (43 - 55% moisture) and soft (>55% moisture) ripened and unripened cheeses produced from milk derived from the main commercial dairy species of cow, sheep and goat. Cheeses were selected which would encompass a range of styles within each specified moisture category. A further analysis was undertaken to determine if it was possible to apply the findings of the risk assessments to other cheeses which lie within the same moisture category.

The key determinant for the safety of raw milk cheese is the microbiological quality of the raw milk. Although the cheesemaking process for some cheeses will compensate for the inherent microbiological risks associated with raw milk, for other cheeses the cheesemaking process will either have no effect or may exacerbate these risks. The main findings of the risk assessments can be summarised as follows:

- For the general population:
    - The selected extra hard raw milk cheeses were all assessed to pose a low to negligible risk to public health and safety as survival and growth of *Campylobactyer jejuni/coli*, enterohaemorrhagic *Escherichia coli* (EHEC), *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* is very unlikely.
    - The selected Swiss-type raw milk cheeses were all assessed as posing a low to negligible risk to public health and safety for the general population as survival and growth of *C. jejuni/coli*, *E. coli* (EHEC), *Salmonella* spp. and *S. aureus* is very unlikely.
    - The modelled raw milk Cheddar cheese was assessed as posing a high risk to all population groups due to the survival and growth of pathogenic *E. coli* during cheesemaking.
    - The overall risk to public health and safety posed by the modelled raw milk blue cheese was unable to be ascertained due to a lack of data.
    - The modelled raw milk Feta cheese was assessed as having a high risk to public health and safety to all population groups due to the survival of pathogenic *E. coli* during cheesemaking.
    - The modelled raw milk Camembert cheese was assessed as having a high risk due to the survival and growth of pathogenic *E. coli*.

- For susceptible populations:
    - Raw milk Swiss-type cheeses with a low curd cooking temperature, blue, Feta and Camembert cheese pose a high risk to public health and safety to susceptible populations due to the survival and/or growth of *L. monocytogenes* during cheesemaking.
- Extrapolation of the findings of the raw milk extra hard cheeses and modelled Camembert assessed may be applied to the extra hard cheese (<36%) and soft (>55%) moisture categories, respectively. However, the ability to apply the findings on the raw milk Swiss, modelled Cheddar, blue and Feta cheeses, to assess the safety of other cheeses within the same moisture category, was variable.
- The survival or inactivation of pathogens in cheese is dependent upon a complex interaction of many intrinsic and extrinsic factors. The lack of key pieces of data and the variability in available data highlights the difficulties in providing information on the risks associated with broad classes or categories of cheese.
- The factors during cheesemaking which have the greatest impact upon the microbiological safety of the raw milk cheeses evaluated include the:
    - Microbiological quality of the raw milk
    - Acidification step
    - Temperature and duration of curd cooking
    - Temperature and duration of maturation

Foodborne illness has been linked to the consumption of cheese; however 70% of all cheese implicated in foodborne illness outbreaks are raw milk cheeses. The presence of EHEC, *Salmonella* spp., *Brucella* spp. and *L. monocytogenes* in raw milk cheeses are responsible for the majority of these outbreaks, with cheeses with high moisture content (*e.g.* soft and fresh cheeses) those most often implicated.

Paramount to the safety of all raw milk cheeses is the microbiological quality of the raw milk.

The primary source of contamination in raw milk cheese is from the raw milk itself, as the milk does not receive a pathogen elimination process such as pasteurisation. Other sources of contamination are the cheesemaking environment including equipment, personnel or cross-contamination between finished products and raw materials. These sources of contamination apply equally to both pasteurised and raw milk cheeses.

The ability of pathogens to survive and/or grow in cheese is largely dependent on: the manufacturing steps during cheesemaking (extent of acidification by the starter culture, the amount of heat applied at various stages during the manufacture, ripening/maturation conditions); the physicochemical characteristics of the cheese (pH, salt content, water activity); and the growth requirements of the microorganism.

Critical for minimising the growth of pathogens in all raw milk cheeses is reaching the appropriate end point pH during acidification. In addition to acid production, starter cultures also contribute to the safety of cheese through competitive inhibition and production of various antimicrobial compounds. The role that curd cooking and maturation play in ensuring the safety of various raw milk cheeses differs according to the specific cheese type. The greatest lethal effect on pathogens is achieved through the application of heat, either to the

raw milk or the cheese curd (*e.g.* pasteurisation, thermisation and curd cooking). This process ranges from being bacteriostatic in low curd cooked cheeses to bacteriocidal for high curd cooked cheeses. Pathogen die-off (inactivation) achieved during the ripening period is also extremely variable and depends upon the specific physicochemical characteristics of the cheese and the properties of the microorganism.

In summary, cheesemaking involves a combination of hurdles that influence the growth and survival of pathogenic microorganisms. It is this combination of hurdles rather than an individual processing step or physicochemical property that has the greatest impact on pathogen survival in a given raw milk cheese.

The risk of selected microbiological hazards to public health and safety from the consumption of various cheeses was characterised using a qualitative framework. The qualitative framework categorises the risk for each microbiological hazard (based on severity of illness and infective dose) with exposure information (raw milk contamination and effect of processing). Prevalence data, where available, was used to determine raw milk contamination while the fate of selected pathogens during cheesemaking was assessed and used to determine the effect of processing. When combined, an estimate of risk can be obtained.

The qualitative framework inputs for 'severity' and 'infective dose' for each pathogen are predetermined and do not vary between cheeses. However, changing input values for 'raw milk contamination' and/or 'effect of processing' will impact upon the final estimate of risk.

The fate of *E. coli*, *S. aureus* and *L. monocytogenes* during the production of raw milk Cheddar, blue, Camembert, and Feta style cheese (*i.e.* effect of processing) was assessed using quantitative models developed by the University of Tasmania and adapted by Food Standards Australia New Zealand (FSANZ). The fate of *E. coli, S. aureus, L. monocytogenes, Salmonella* spp. and *Campylobacter* spp. in raw milk extra hard and Swiss-type cheeses was assessed qualitatively.

Using the qualitative framework, the principal risks to public health and safety from the consumption of raw milk cheeses are summarised in Table 1.

Table 1: Principal risks from consumption of specific raw milk cheeses

Hazard	Extra Hard	Swiss	Cheddar	Blue	Feta	Camembert
C. jejuni	Negligible	Negligible	NA	NA	NA	NA
***E. coli* (EHEC)**	Low	Low	High	NA	High	High
***Salmonella* spp.**	Negligible	Negligible	NA	NA	NA	NA
S. aureus	Negligible	Negligible	Very Low	NA	Low	Low
L. monocytogenes	Negligible Very low[#]	Negligible/ Very low[#] Low/High[1,#]	Negligible Low[#,2]	Low High[#]	Low High[#]	Low High[#]

\# Susceptible populations NA Not assessed
1 Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine 2 Moderate if made from sheep milk

The source of the raw milk (cow, goat or sheep) was not found to significantly impact upon the safety of the modelled cheeses, with the exception of *L. monocytogenes* in raw milk Cheddar cheese. *L. monocytogenes* presents a greater risk in Cheddar produced from raw sheep milk, due to its reported higher prevalence in raw sheep milk, compared to cow and goat milk. Prevalence of microbiological hazards in raw milk can impact on the estimated level of risk.

A summary of the net change in the predicted modelled growth or inactivation of *E. coli, S. aureus* and *L. monocytogenes* in raw milk Cheddar, blue, Feta and Camembert cheeses is illustrated in Figure 1. This reflects changes over the course of cheese manufacture, with processing affecting pathogen growth, survival or inactivation and impacting upon the estimated level of risk.

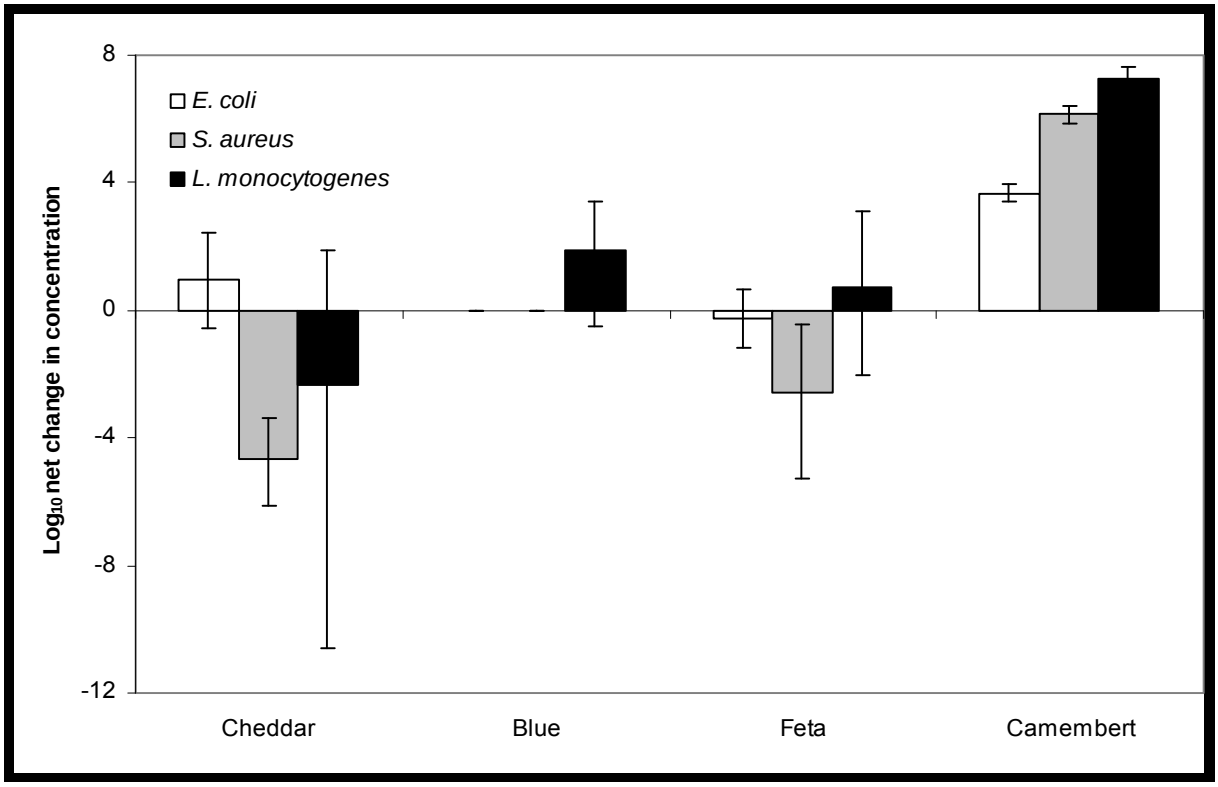

Figure 1: Net change (log_{10} CFU/g) in the predicted concentration of *E. coli, S. aureus* and *L. monocytogenes* in raw milk Cheddar, blue, Feta and Camembert cheeses. The bars are the predicted mean change. The error bars indicate the 5th and 95th percentile values.

Rates of pathogen inactivation during ripening/maturation in the quantitative modelling were based on results from published challenge studies. Statistical analysis of these studies highlighted the high variability between strains and also between trials for the same strain, particularly for *L. monocytogenes* in Cheddar cheese (illustrated as error bars in Figure 1).

The inclusion of a lag phase in the model and the inhibition of growth due to rapid pH decline during acidification would reduce the difference between the reported studies and the model predictions. However the inclusion of a lag phase will have less effect on the final concentration in the cheese compared to the inhibition of pathogen growth during acidification.

Quantitative models were used to estimate the maximum pathogen load which could be present in the incoming raw milk which would permit raw milk Cheddar, blue, Feta and Camembert cheeses to be made which are compliant with the *Australia New Zealand Food Standards Code* (the Code). The initial pathogen concentrations are listed in Table 2.

Table 2: Initial concentration in raw milk required to meet current microbiological limits in the Code

Pathogen	Cheddar	Blue	Feta	Camembert
E. coli	< 0.01 cfu/ml	n/a	<1 cfu/ml	$<10^{-3}$ cfu/ml
L. monocytogenes	$< 10^{-3}$ cfu/ml	$<10^{-5}$ cfu/ml	$<10^{-5}$ cfu/ml	$<10^{-7}$ cfu/ml
S. aureus*	<100 cfu/ml	n/a	$<10^{3}$ cfu/ml	$<10^{-4}$ cfu/ml

* Initial numbers to ensure numbers do not reach levels that may produce enterotoxin to cause illness (*i.e.* $<10^{5}$ cfu/g) as there is no limit for *S. aureus* in the Code.

Consumption data on raw milk cheeses is unavailable in Australia. Cheese production statistics indicate hard and semi-hard cheeses account for 75% of Australia's cheese production, whereas soft and blue style cheeses account for less than 1% of production. Consumption of extra hard, Swiss, blue, Feta and Camembert/Brie cheeses during the National Nutrition Survey (NNS) was extremely low, whereas Cheddar cheese was the most commonly consumed cheese . Changes in dietary habits over the past ten years would suggest Australians readily source and consume a range of new and exotic foods, which could well include specialty raw milk cheeses if they were available.

Data gaps identified in the risk assessment include:

- The incidence and prevalence of pathogens in raw cow, goat and sheep milk in Australia
- The effect of processing - qualitative assessment used to determine the fate of various pathogens during cheesemaking
    - Limited information on the individual cheesemaking processes
    - Limited details on physicochemical properties of individual cheeses
    - Limited challenge study data
- The effect of processing - quantitative modelling used to determine the fate of various pathogens during cheesemaking
    - Growth/inactivation rates between different strains of the same organism
    - Effect on pathogens of changing physicochemical properties during cheese maturation
    - Explicit consideration of the effect of lactic acid on pathogens not included for some pathogen:cheese pairings
    - Effect of competitive microflora
    - Inclusion of lag phase models

Further information on the above would reduce the amount of uncertainty in the levels of estimated risk for the various raw milk cheeses assessed. Assumptions made in this risk assessment to bridge these gaps were conservative which results in overall protective estimates of risk.

Conclusions

The safety of raw milk cheese is dependent upon a range of hurdles that influence the presence, growth, survival and inactivation of pathogenic microorganisms. For example, variations in salt content along with water activity in a specific cheese will impact on the extent to which a pathogen may survive or grow. Similarly, the duration and temperature of ripening will affect pathogen survival or growth. The impact these factors have on the fate of

pathogens during cheese manufacture varies significantly between cheesemaking processes and the myriad of types of cheese.

The risk assessment highlighted the difficulty in evaluating the safety of raw milk cheeses due to the lack of suitable data and variability of data which is available. Variability and uncertainty have been included where possible in the evaluation to assess the fate of pathogens during cheese production. However, safety assessments of raw milk cheese require detailed information on the specific manufacturing process, physicochemical characteristics of the cheese and challenge data.

Not surprisingly, these factors impact on the capacity to apply the findings for the specific cheeses evaluated to assess the safety of other cheeses within the same moisture category (see Table 3). While the cheeses assessed are examples of very hard, hard, semi-soft and soft cheese based on moisture content, they are not necessarily representative of all cheeses found within these categories. For example, the modelled blue cheese may be considered a semi-soft cheese when classified on moisture content, but not all semi-soft cheeses are mould ripened (*e.g.* Brick, Edam and Gouda). In addition, subdivision of cheeses based on moisture can be arbitrary and overlapping. While cheeses are often grouped according to moisture content, the cheeses which are grouped together may differ widely in physicochemical characteristics, both of the curd and the final cheese, and manufacturing protocols (*e.g.* Cheddar, Parmesan and Emmentaler are often grouped together as hard cheeses).

Table 3: Comparison of risk assessment findings to cheese types

Raw milk cheese assessed	Moisture category	Findings applicable to moisture category	Findings applicable to cheese type	Comments
Parmigiano Reggiano Grana Padano Romano Asiago Montasio Sbrinz	Extra hard (<36%)	Applicable	Applicable	The cheeses assessed are likely to represent other cheeses in the extra hard moisture category. Extra hard cheeses generally have similar physicochemical characterises and manufacturing protocols *e.g.* curd cooking and long ripening times.
Emmentaler Gruyère Appenzeller Tilsiter Vacherin Fribourgeois Tête de Moine	Hard (37 - 42%)	Not applicable	Applicable (Internal bacterially ripened cheese with eyes - lactate)	The moisture contents of the assessed Swiss-type cheeses overlap between the extra hard and hard moisture categories (31 - 44%) and are not representative of all hard cheeses. This group of bacterially ripened cheeses with eyes has different physicochemical characteristics and manufacturing protocols to other hard and extra hard cheeses.
Cheddar	Hard (37 - 42%)	Not applicable	Applicable (Internal bacterially ripened hard cheese)*	Cheddar is a milled, dry-salted cheese having different physicochemical characteristics and manufacturing protocols to other hard cheese, and therefore does not represent all hard cheese.
Blue	Semi-soft (43 -5 5%)	Not applicable	Applicable (Mould ripened –internal mould cheese)*	Moisture contents of blue cheeses vary and can overlap between moisture categories from soft to semi-soft/semi-hard. The physicochemical characteristics of other cheeses within the mould ripened (internal mould) category are also variable.
Feta	Semi-soft (43 - 55%)	Not applicable	Applicable (Internal bacterially ripened high salt variety)*	Feta cheese is not representative of all semi-soft cheeses. This high salt variety has very different physicochemical characteristics and manufacturing protocols to other semi-soft cheeses.

Table 3 cont: Comparison of risk assessment findings to cheese types

Raw milk cheese assessed	Moisture category	Findings applicable to moisture category	Findings applicable to cheese type	Comments
Camembert	Soft (>55%)	Applicable	Applicable (Mould ripened – surface mould cheese)*	Camembert cheese is likely to represent other cheeses in this same moisture category as cheeses in this category generally have similar physicochemical characteristics and manufacturing protocols *e.g.* minimal curd cooking, high moisture content and short ripening times.

* Cheeses whose manufacturing parameters lie within the range of those of the modelled raw milk cheese

2. Background

Food Standards Australia New Zealand (FSANZ) has responsibility for protecting the health and safety of consumers through the development of food standards.

A comprehensive analysis to identify and examine microbiological hazards along the entire dairy supply chain was conducted by FSANZ and published as a Risk Profile in 2005[1]. One of the key findings of the Profile was that Australian dairy products have an excellent reputation for food safety. This is because dairy products in Australia are made from pasteurised milk, and pasteurisation represents the principal process for rendering dairy products safe for consumption. This finding was supported by the lack of evidence attributing foodborne illness to dairy products.

Although the Profile confirmed that unpasteurised dairy products are the most common cause of dairy associated foodborne illness, it did not specifically examine risks to public health and safety from consumption of raw milk cheeses. This document seeks to assess the risk to public health and safety resulting from consumption of selected raw milk cheeses.

FSANZ uses a number of tools to assess risks to public health and safety, including risk profiling[2], quantitative and qualitative risk assessments[3] and scientific evaluations. The application of these tools to the assessment of the risks to public health and safety is dependent on the purpose of the assessment and on the availability, quality and quantity of relevant data.

FSANZ follows established international guidelines and incorporates elements of the Codex Alimentarius Commission risk assessment framework when undertaking risk profiles, risk assessments and other scientific evaluations. Guidance for undertaking risk assessments have been drafted internationally by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

When assessing risks to public health and safety, available scientific data concerning the safety of the commodity under consideration and the properties of the hazard are evaluated. This requires utilisation of relevant scientific data and includes procedures to address uncertainty and variability in the conclusions drawn from the data *i.e.* consideration of the relevance and quality of data and the veracity of its source.

The outcome of any assessment of risks to public health and safety may include a statement on the probability and severity of an adverse health effect due to the consumption of a food containing a particular biological, chemical or physical agent. An assessment may also identify where in the production chain controls over hazards will have the greatest impact on minimising risk *i.e.* informing risk managers where intervention will be most effective. The outcomes of this risk assessment may be used by FSANZ to inform risk management decisions.

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1 *A Risk Profile of Dairy Products in Australia*: http://www.foodstandards.gov.au/_srcfiles/DAR_P296_Dairy_PPPS_Attach2%20Parts%20A-B.pdf#search=%22Risk%20Profile%22

2 Risk profiling is defined by FAO/WHO as 'the process of describing a food safety problem and its context, in order to identify those elements of the hazard or risk relevant to various risk management decisions'.

3 Risk assessment is defined by Codex as "a scientific process undertaken to characterise the risk to public health and safety posed by foodborne hazards associated with a food commodity".

3. Purpose and scope

3.1 Purpose

The purpose of this risk assessment is to provide an objective interpretation of available scientific data on the public health risks associated with the consumption of raw milk cheeses and to examine the impact of processing steps on the safety of raw milk cheeses.

The assessment of the public health and safety risks posed by consumption of raw milk cheese was undertaken to address the following overarching questions:

(1) What are the risks to public health and safety posed by the consumption, in Australia, of raw milk cheese?

(2) What are the factors that would have the greatest impact on public health and safety along the production chain for raw milk cheese?

3.2 Scope

In order to assess the public health and safety of raw milk cheese, the scope of the risk assessment was to evaluate very hard (<36% moisture), hard (37 - 42% moisture), semi-soft (43 - 55% moisture);and soft (>55% moisture) ripened and unripened cheeses on a moisture basis[4], from the main commercial dairy species of cow, sheep and goat.

3.3 Definition of raw milk cheese

Codex defines **raw milk**[5] as "milk which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect". Similarly, European Union (EU) Directives define raw milk as "milk produced by secretion of the mammary glands of one or more cows, sheep, goats, or buffaloes from a single holding that has not been heated beyond 40°C or undergone any treatment having a similar effect" (as defined in Codex General Standard for the Use of Dairy Terms[6]).

The *Australia New Zealand Food Standards Code* (the Code)[7] specifies processing temperatures for pasteurisation and thermisation in relation to milk. Therefore "raw milk" for the purposes of this assessment is defined as milk which has not been heat treated in accordance with the Code.

Use of the term "raw milk" rather than "unpasteurised milk" recognises that there are processes other than pasteurisation which are currently permitted *e.g.* thermisation.

For the purposes of this assessment, a cheese produced with milk which meets the above definition of "raw milk" is deemed to be a raw milk cheese.

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4 Moisture content parameters are a combination of the Codex Extra Hard Grating Cheese Standard (Codex STAN C-35-1978) and the classification system of Burkhalter (1981) as contained in Fox *et al.* (2000)

5 Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004)

6 Codex General Standard for the Use of Dairy Terms (CODEX STAN 206-1999)

7 The Australia New Zealand Food Standards Code - Standard 1.6.2 – *Processing Requirements*

3.4 Approach

The risk assessment qualitatively examines specific microbiological hazards, epidemiological evidence and other relevant data to determine (a) whether these hazards have presented, or are likely to present a public health risk, and (b) to identify where in the cheesemaking process these hazards may be introduced and/or their levels change. The assessment draws upon findings of the Profile for information relating to milk production and utilises available information including current scientific and epidemiological data and challenge studies.

Specifically the assessment:

- Identifies microbiological hazards of public health significance in raw milk cheese
- Identifies the risk factors that may impact on the likelihood of raw milk cheeses becoming contaminated with microbiological hazards during processing, and the relative importance of these factors
- Examines the impact of processing steps during cheese manufacture on microbiological hazards
- Includes probabilistic models to determine the fate of *E. coli, S. aureus* and *L. monocytogenes* in raw milk Cheddar, blue, Feta and Camembert style cheese
- Qualitatively evaluates the public health and safety risks due to significant pathogens associated with raw milk cheeses

Codex have established an internationally recognised framework for undertaking a microbiological risk assessment[8]. The risk assessment process used by FSANZ is consistent with international protocols and involves four distinct steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation.

There is no internationally agreed framework for undertaking a qualitative risk assessment for microbiological hazards. While Codex and FSANZ[9] have guidelines for conducting microbiological risk assessments but they do not provide actual tools that can be used to objectively assess or rank the risk to public health and safety. In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles and employs elements of Risk Ranger (Ross and Sumner, 2002), a widely accepted semi-quantitative tool for the assessment food safety risks (Appendix 1).

3.4.1 Selection of cheeses

A suitable approach given the scope of this risk assessment was to assess selected cheeses that would encompass a range of styles within each specified moisture category. A further analysis would then be undertaken to determine the possibility of applying the findings to other cheeses within the same moisture category.

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8 CODEX (CAC/GL 30, 1999) Principles and Guidelines for the Conduct of Microbiological Risk Assessment http://www.codexalimentarius.net/download/standards/357/CXG_030e.pdf

9 FSANZ (2009) The Analysis of Food-Related Health Risks. http://www.foodstandards.gov.au/_srcfiles/Food%20Related%20Health%20Risks%20WEB_FA.pdf

The cheeses selected for this risk assessment represent a broad sample set across a range of moisture contents and ripening characteristics. The selection of cheeses was based on:

- The requirement to assess cheeses within specified moisture content ranges (*i.e.* < 36%; 37 - 42%; 43 - 55%; and > 55%)
- The need to cover different manufacturing protocols *e.g.* varying coagulation methods and ripening conditions
- The availability of suitable data
- Knowledge of specific processing parameters
- Raw milk cheese varieties which are manufactured and traded internationally

For cheeses within the extra hard moisture category (>36%) Parmigiano Reggiano, Grana Padano, Pecorino Romano, Asiago, Montasio and Sbrinz were selected. These cheeses span different styles within the internal bacterially ripened extra hard moisture category and for the purposes of this risk assessment are collectively referred to as raw milk extra hard cheeses. The assessment of raw milk extra hard cheeses draws upon the risk assessments undertaken by FSANZ during the evaluation of Proposal P263 (Safety assessment of raw milk very hard cooked-curd cheeses)[10] and Application A357 (Swiss raw milk cheeses)[11].

Similarly, the raw milk Swiss-type cheeses Emmentaler, Gruyère, Appenzeller, Tilsiter, Vacherin Fribourgeois and Tête de Moine were assessed during the evaluation of Application A357 (Swiss raw milk cheeses). These cheeses represent a range of internal bacterially ripened cheese with eyes (lactate fermentation) and overlap the extra hard and hard moisture categories (34 - 44%). Consequently, this group of cheeses have been specifically considered in the risk assessment and are collectively referred to as Swiss-type raw milk cheeses.

Cheddar cheese is the most common internal bacterially ripened hard cheese and was therefore selected as an example of a hard cheese based on moisture (37 - 42%). The 'semi-soft' category of cheese (43 - 55%) can sometimes be referred to as 'semi-hard' and as such two cheeses were selected in order to encompass a broader range of manufacturing protocols. Blue cheese was chosen as characterising a semi-soft mould ripened (internal mould) cheese, while Feta represents a semi-hard internal bacterially ripened high-salt variety cheese. Camembert was selected as an example of a soft (>55%) mould ripened (surface ripened) cheese.

For all cheeses selected, the risk to public health and safety was assessed on the basis of cheeses being produced using raw milk from cow, goat or sheep species.

3.4.2 Qualitative framework

The qualitative framework considers the characteristics of identified hazards (hazard identification and characterisation) and an assessment of the likely exposure to these hazards (exposure assessment) to arrive at a final estimate of risk (risk characterisation).

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10 Proposal P263 – Safety assessment of raw milk very hard cooked-curd cheesses - http://www.foodstandards.gov.au/_srcfiles/P263rawcheeseFAR.pdf

11 Application A357 – Swiss Raw Milk Cheeses http://www.foodstandards.gov.au/_srcfiles/A357%20FAR.pdf

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of the disease. The exposure module considers the likelihood of the hazard being present in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of risk.

Essentially, the framework categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

3.4.2.1 Input parameters

3.4.2.1.1 Hazard characterisation module

The hazard characterisation module combines information on the infective dose of the microorganism and the severity of illness which may result for certain population groups. Infective dose information for each microorganism has been derived from published dose response information where available. The module employs elements of Risk Ranger (Ross and Sumner, 2002) and utilises International Commission on Microbiological Specifications for Foods classifications (ICMSF, 2002) for the severity of foodborne illness caused by selected pathogens. The descriptors used in the framework are an amalgamation of information from these sources, combined with expert elicitation and evidence from epidemiological investigations (Appendix 1: Tables 1, 2 and 3).

The inputs for the hazard characterisation module remain the same regardless of the type of cheese being assessed (*e.g.* raw milk extra hard or raw milk blue cheese) or the origin of the milk (*e.g.* cow or goat).

Assumptions used in the framework, including infective dose, severity of hazards, and the likely levels of pathogens in raw milk, are given in Appendix 1. Information used to derive these assumptions included scientific data, published literature and expert elicitation.

3.4.2.1.2 Exposure module

The exposure module combines information on the likely level of the hazard in the raw milk (prevalence data) and the effect of processing on the hazard.

Contamination of raw cow, goat and sheep milk by pathogens was determined from international and domestic prevalence data, scientific literature and expert elicitation (Appendix 1: Tables 5, 6 and 7). This information was then assigned to a category in the qualitative framework according to "best fit" to qualifying parameters (Appendix 1: Table 4). For example, a 10% prevalence was categorised as 'sometimes'. Using a different raw milk contamination input parameter (*e.*g milk from different species with an 'infrequent' parameter) when assessing a specific raw milk cheese may change the resulting risk characterisation outcome for that particular cheese.

Two different approaches (qualitative and quantitative) were used to determine the effect of cheesemaking on selected pathogens in the various cheeses. Results were then used to describe the "effect of processing" input parameter in the qualitative framework.

For the manufacture of raw milk extra hard and Swiss-type cheeses, data to assess the fate of microbiological hazards during the cheesemaking process has been determined qualitatively. This was based on scientific evaluations previously performed by Food Science Australia for FSANZ. These evaluations were used during the assessments of Application A357[12] and Proposal P263[13]. This data was then allocated an input according to the "best fit" to qualifying definitions. For example, in raw milk extra hard cheese, *E. coli* was assessed to receive a 5 log reduction during curd cooking and a further 5 log reduction during ripening. This was assigned to an "Eliminates" determination for the effect of processing input parameter.

Quantitative models were developed by the University of Tasmania and adapted by FSANZ to determine the fate of *E. coli*, *S. aureus* and *L. monocytogenes* during the production of raw milk Cheddar, blue, Camembert, and Feta style cheese.

Quantitative models

To simulate the fate of microorganisms during the production of a number of different cheeses, mathematical models were developed using existing growth/inactivation models and information on the properties of selected cheeses during manufacture. The cheeses modelled were raw milk Cheddar, blue, Feta and Camembert. To account for uncertainty and variability in the model, probability distributions were incorporated using @Risk (Palisade Corporation, New York).

The growth rate of bacteria in the cheese curd/whey mix is neither static nor wholly dependent on temperature. There are several physical and chemical changes which occur that influence the growth rate of organisms during the manufacture of cheese. The most significant of these, excluding temperature changes, is the addition of a starter culture to the milk which results in an increase in lactic acid concentration, reduction in pH and production of antagonistic compounds. Of these, only the pH reduction has been included in the model. Similarly, lag phase models have not been included in the mathematical model due to a lack of adequate data. The presence of a lag phase would further minimise bacterial growth and reduce any estimated exposure. Model estimates are therefore conservative in nature. Unless stated otherwise, the same growth models were used for each cheese type and are provided in Appendix 2.

Several initial pathogen contamination concentrations in milk entering the cheese production process were modelled, with values ranging between 0.001 - 100 cells/ml of pathogens in the raw milk.

The data obtained from these models were then assigned to an "effect of processing" input category in the qualitative framework according to "best fit". For example, in raw milk Feta cheese the modelling determined that *E. coli* had a net log reduction of 0.27, whilst *L. monocytogenes* had a net increase of 0.70 log. Allocation of "effect of processing" parameters results in *E. coli* having a "50% reduction" and *L. monocytogenes* a "10 fold increase" input for raw milk Feta cheese. In assigning the "effect of processing" input category it must be recognised that the net log changes are based on the mean model outputs and consideration of estimated variability (*e.g.* between the 5th and 95th percentile estimates).

[12] Application A357 – Swiss Raw Milk Cheeses - http://www.foodstandards.gov.au/_srcfiles/A357%20FAR.pdf

[13] Proposal P263 – Safety assessment of raw milk very hard cooked-curd cheesses - http://www.foodstandards.gov.au/_srcfiles/P263rawcheeseFAR.pdf

In determining the risk from a particular pathogen, the "effect of processing" parameter may differ between raw milk cheeses depending on the fate of that pathogen during the cheesemaking process and ultimately this affects the risk characterisation.

3.4.2.2 Example

A detailed example of how the qualitative framework was utilised to characterise the risk from Enterohaemorrhagic *Escherichia* coli (EHEC) in raw milk extra hard cheese produced from raw cow milk in the general population is given in Appendix 1.

Briefly, the hazard characterisation for EHEC in this specific cheese is high combining a "low" infective dose (<10) with a "serious" consequence of exposure in the general population. The exposure assessment was rated as negligible due to the "infrequent" product contamination combined with "elimination" of *E. coli* during processing. Combining the hazard characterisation and exposure assessment results gives EHEC in cow raw milk extra hard cheese a risk characterisation of **low** for the general population.

Changing inputs for 'raw milk contamination' and/or 'effect of processing' may impact upon the final estimate of risk. For example, the risk to public health and safety from EHEC in raw milk Feta cheese was assessed as **moderate** when produced from either raw cow or raw goat milk. When using raw sheep milk, the risk was assessed as **high** as the raw milk contamination parameter is higher for sheep milk than for raw cow or goat milk.

3.4.2.3 Modelling the initial contamination in raw milk

The quantitative model developed by the University of Tasmania and adapted by FSANZ was also run in a retrospective fashion to estimate the concentration of pathogens in raw milk that would result in a finished cheese that will meet the microbiological limits in Standard 1.6.1 of the Code.

3.4.3 Uncertainty and variability

In characterising the risk associated with consuming raw milk cheeses in Australia, the level of confidence in the final estimate of risk is influenced by the adequacy and quality of the available data. Variability is associated with biological systems, food processing technologies, food preservation methods and human behaviour and is therefore inherent in these types of assessments. Uncertainty relates to assumptions which had to be made due to a lack of information. Details of the assumptions used in the qualitative framework are contained in Appendix 1.

Qualitative framework

There was a degree of uncertainty in components of the qualitative framework due to lack of available data. In particular there was a lack of information on:

- The incidence and prevalence of pathogens in raw cow, goat and sheep milk in Australia
- The severity of illness within certain population groups;
- The effect of processing

Where data was not available, gaps were bridged using expert elicitations involving members of the Dairy Scientific Advisory Panel. Elicitations to determine model inputs were primarily

for the severity of illness in general and susceptible populations and initial contamination levels in the raw cow, goat and sheep milk. Assumptions are detailed in Appendix 1.

There is a high level of uncertainty in the model due to the assumptions used. More data, particularly on the incidence and prevalence of pathogens in raw milk in Australia would reduce the level of uncertainty and improve confidence in the outputs.

The exposure assessment module characterises exposure to the hazard based on the likely level of the hazard in the initial raw product and the effect of processing on the hazard. Significant variability exists within the processes used for the production of raw milk cheeses. The processing steps vary widely between cheese types, and within the same cheese type, plus the cheesemaker will subtly vary the way each batch of cheese is prepared, reflecting seasonal and other variations in milk properties, starter culture fecundity, etc. Variations in times and temperatures used for curd cooking, salt addition, brine concentrations, maturation times and temperatures occur between individual cheeses, even within the same cheese type *e.g.* manufacturing processes for the various Swiss-type cheeses vary significantly.

Quantitative models

There was a degree of uncertainty in components of the quantitative models due to lack of available data on factors such as:

- Growth and/or inactivation rates between different strains of an organism
- Effect of changing cheese physicochemical properties on pathogens during cheese maturation
- Effect of lactic acid concentrations on the growth of some pathogens
- Inhibitory effect of competitive microflora
- Presence of an initial lag phase on growth of pathogenic microorganisms at the start of the cheesemaking process
- Growth and no growth boundaries

Data on the variability in growth and/or inactivation between strains of pathogens was included in the model where available. For example, challenge studies demonstrated there is a large variation in inactivation rates between strains of *L. monocytogenes* during the ripening of cheese. One consequence of this variability is the wide range in the predictions for the concentration at the end of ripening/maturation. In the case of the Cheddar cheese, the quantitative model 5^{th} and 95^{th} percentile values for the net change in concentration for *L. monocytogenes* ranged across 12-orders of magnitude. Only the mean value was used in the qualitative framework.

It would be beneficial to have more specific information on the strains most likely to be encountered in a cheese production facility, especially those associated with cheese-borne illness, as this would have a bearing on the inactivation kinetics during ripening and reduce the overall uncertainty in the output.

Variation during each of the processing steps was described by probability distributions in the model. In this respect, the modelling approach attempts to cover the range of potential time and temperature combinations during manufacture that may be used to produce each specific cheese. Insufficient data was available to model the effect on pathogens of all the continually changing physicochemical parameters of cheese during maturation.

The inclusion of a lactic acid term in the model makes a significant difference to the growth rate estimates and ultimately the final concentration of the microorganism in cheese (Murphy, 1996). For the potential growth of pathogens during cheese production, there was little information regarding the development of lactic acid during fermentation. Consequentially, the *L. monocytogenes* growth model used for the Cheddar, Feta and blue cheese does not incorporate a lactic acid term. A second growth model for *L. monocytogenes* has been implemented that accounts for the lactic acid concentration in the Camembert cheese only (Ross and Soontranon, 2006). This model has the same functional form as the *E. coli* growth model (Ross *et al*., 2003) used for the Cheddar, Feta and Camembert cheeses. This second growth model for *L. monocytogenes* provides more realistic estimates of *L. monocytogenes* concentrations at the end of cheese maturation. There are no known growth rate models available that describe the effect of lactic acid on *S. aureus*. Inclusion may reduce the estimates of the final concentration of the pathogen in all cheeses modelled.

An aspect that was not included in the modelling was the effect of competition on the growth of the three pathogens. It was assumed that they are unrestrained in their growth potential during the manufacture of the cheese. However, given the relatively small numbers of pathogens in the milk compared, for example, with the starter culture, there exists the possibility of a reduced pathogen growth rate due to competitive exclusion (Breidt and Fleming, 1998; Gimenez and Dalgaard, 2004). Although the effects of competition between pathogenic bacteria and starter culture bacteria have not been modelled, the impact of resultant pH changes resulting from the growth of the starter culture is included.

Lag phase models were not included as an explicit step in the modelling process due to a lack of available data. Addition of a lag phase would reduce the predicted growth of pathogens during the initial phase of cheese manufacture.

Consideration of the inhibition of growth due to rapid pH decline during acidification was not included in the model due to the complexity of describing the temporal changes in physicochemical characteristics of the cheese and a lack of quality data. Inclusion of these factors in model would reduce the total predicted concentration of pathogens at the end of production.

3.5 Other raw milk cheese assessments

There have been few microbiological risk assessments undertaken for raw milk cheeses. Assessments include those pertaining to *L. monocytogenes* in raw milk soft cheese, Swiss Emmental cheese and ready-to-eat foods including cheese; *S. aureus* in raw milk cheese; and Enterococci in Irish artisanal cheese and Italian cheeses.

Reviews on the safety of raw milk cheese have been conducted by a number of authors (Johnson *et al*., 1990b; Johnson *et al*., 1990c: Eyles, 1992: Donnelly, 2001: DRINC, 2006), while comprehensive reviews have also been published regarding outbreaks of human illness linked to the consumption of cheese (Altekruse *et al*., 1998; Johnson *et al*., 1990a). Numerous challenge studies examining the survival of various pathogens during the manufacture and ripening of different cheeses have also been published (refer to Section 9.3).

The New Zealand Food Safety Authority is currently undertaking risk assessments on Shiga toxin producing *E. coli* (STEC) in raw bovine milk and boutique cheeses made from bovine,

ovine and caprine milk and *L. monocytogenes* in soft and semi-hard cheeses. The authority is also conducting a risk assessment to assess the safety of raw milk and raw milk products.

Both the New Zealand Food Authority and FSANZ have undertaken assessments looking at the safety of Roquefort cheese from France.

In 1998 the United States Institute of Food Science and Technology (IFST) examined the hazards to human health due to the potential presence of pathogenic bacteria in cheeses made from unpasteurised milk, particularly soft and semi-soft types.

Health Canada is undertaking a comprehensive review to improve the safety of raw foods of animal origin which includes raw milk cheese.

The US Food and Drug Administration Centre for Food Safety and Applied Nutrition is also examining the safety of raw milk cheeses and is developing a risk profile for these cheeses. The Centre is also undertaking a quantitative risk assessment of *L. monocytogenes* in soft cheeses in collaboration with Health Canada.

4. Introduction

Cheese is an ancient food whose origins may predate recorded history. Probably first discovered in Central Asia or the Middle East, cheesemaking then spread to Europe. The exact origins of cheesemaking are debated or unknown, and estimates range from around 8000 BC (when sheep were domesticated) to around 3000 BC.

Cheesemaking likely began as a way of preserving soured and curdled milk through pressing and salting, with rennet introduced later, perhaps when it was noticed that cheese made in an animal stomach produced more solid and better-textured curds.

Cheesemaking remained an art rather than a science until relatively recently. Although the names of many varieties of cheeses have existed for hundreds of years (Table 4), cheeses were not standardised and there may have existed great variation within any one cheese type (Fox, 2004).

Table 4: First recorded date for some major cheese varieties (adapted from Scott (1986) in Fox *et al.,* 2004)

Variety	Year	Variety	Year
Gorgonzola	897	Gouda	1697
Roquefort	1070	Gloucester	1783
Grana	1200	Stilton	1785
Cheddar	1500	Camembert	1791
Parmesan	1579		

There are hundreds of types of cheeses produced all over the world. Different styles and flavours of cheese are the result of using different species of starter bacteria and ripening moulds; different levels of milk fat; differing coagulation methods and processing treatments (cheddaring, pulling, brining, mould wash); variations in length of aging; and using milk from different breeds of cows, sheep, or other mammals. Other factors include variations in animal diet and the addition of flavouring agents such as herbs, spices, or wood smoke.

4.1 Classification of cheeses

Cheese classification schemes have traditionally been based principally on moisture content such as very hard, hard, semi-hard, semi-soft or soft. Classification schemes by Schultz (1952), Davies (1965), Walter and Hargrove (1972), Scott (1986) and Fox (1993) all include moisture content as an important cheese characterising factor. Although many classification systems utilise moisture content as a defining factor, inconsistency exists between category parameters *e.g.* Codex defines soft cheese as >67% moisture on a fat free basis, whereas Schultz (1952) defines soft cheese as 60 – 69.9% moisture content and Scott (1986) and Burkhalter (1981) both employ a limit of >55% moisture.

Even though moisture content is a widely used basis for classification, it suffers from a serious drawback: it groups together cheeses with widely different characteristics and manufacturing protocols *e.g.* Cheddar, Parmesan and Emmental are often grouped together as hard cheeses (Fox *et al.,* 2000). Subdivision of cheeses based on moisture can be arbitrary and overlapping. Most varieties of hard cheese lose moisture throughout ripening *e.g.* Pecorino Romano and Montasio can be consumed throughout ripening and hence may be

classified as semi-hard, hard and extra hard depending on the length of ripening (Fox *et al.,* 2004).

Cheeses may also be grouped according to manufacturing or processing procedures, consistency or rheology (softness or hardness), country of origin, general appearance (size, shape, colour, surface ripening), source of milk, and chemical analysis.

No definitive list of cheese varieties exists, although various attempts have been made to categorise the plethora of cheeses and cheese types available. Sandine and Elliker (1970) suggested there were greater than 1000 different cheese varieties, Path (2006) compiled a list of greater than 1400 cheese varieties (Path, 2006), Walter and Hargrove (1972) described greater than 400 cheese varieties and listed a further 400 varieties and Burkhalter (1981) classified 510 varieties. However, no single categorisation scheme adequately captures the true diversity of cheeses.

Classification systems that have been developed are primarily based on characteristics of the cheese including:

- Texture, which is dependent mainly on moisture content
- Method of coagulation as the primary criterion, coupled with other criteria
- Ripening indices

Full details of major classifications systems can be found at Appendix 3. Unfortunately, none of these classification schemes is completely satisfactory and thus none is universally accepted.

4.2 Principal categories of cheese

Discussion of cheese varieties in this risk assessment follows the modified classification scheme of Fox *et al.* (2000) (Figure 2).

The classification scheme of Fox *et al*. (2000) expanded and modified Fox's original classification scheme (1993), subdividing the rennet coagulated cheeses into further groups based on characteristic ripening agents or manufacturing technology. Fox classifies natural cheese into internally bacterially ripened cheese, mould ripened and surface ripened cheese categories.

The internal bacterially ripened varieties are the most diverse family of rennet coagulated cheeses which is then further subdivided based on moisture (extra hard, hard and semi-hard), the presence of eyes, or a characteristic technology such as cooking/stretching or ripening under brine. Internal bacterially ripened cheese with eyes is further subdivided into hard varieties *e.g.* Swiss type (lactate metabolism) or semi-hard *e.g.* Dutch type (citrate metabolism) types.

Soft cheese varieties are usually not included in the group of internal bacterially ripened cheeses because they have a characteristic secondary microflora which has a major effect on the characteristics of the cheese (Fox *et al.,* 2004). Mould ripened cheeses are subdivided into surface mould *e.g.* Brie and Camembert, and internal mould *e.g.* Roquefort and Stilton.

The Fox *et al*. (2000) classification system is briefly described in Table 5 with more detailed descriptions in Appendix 3: Table 6.

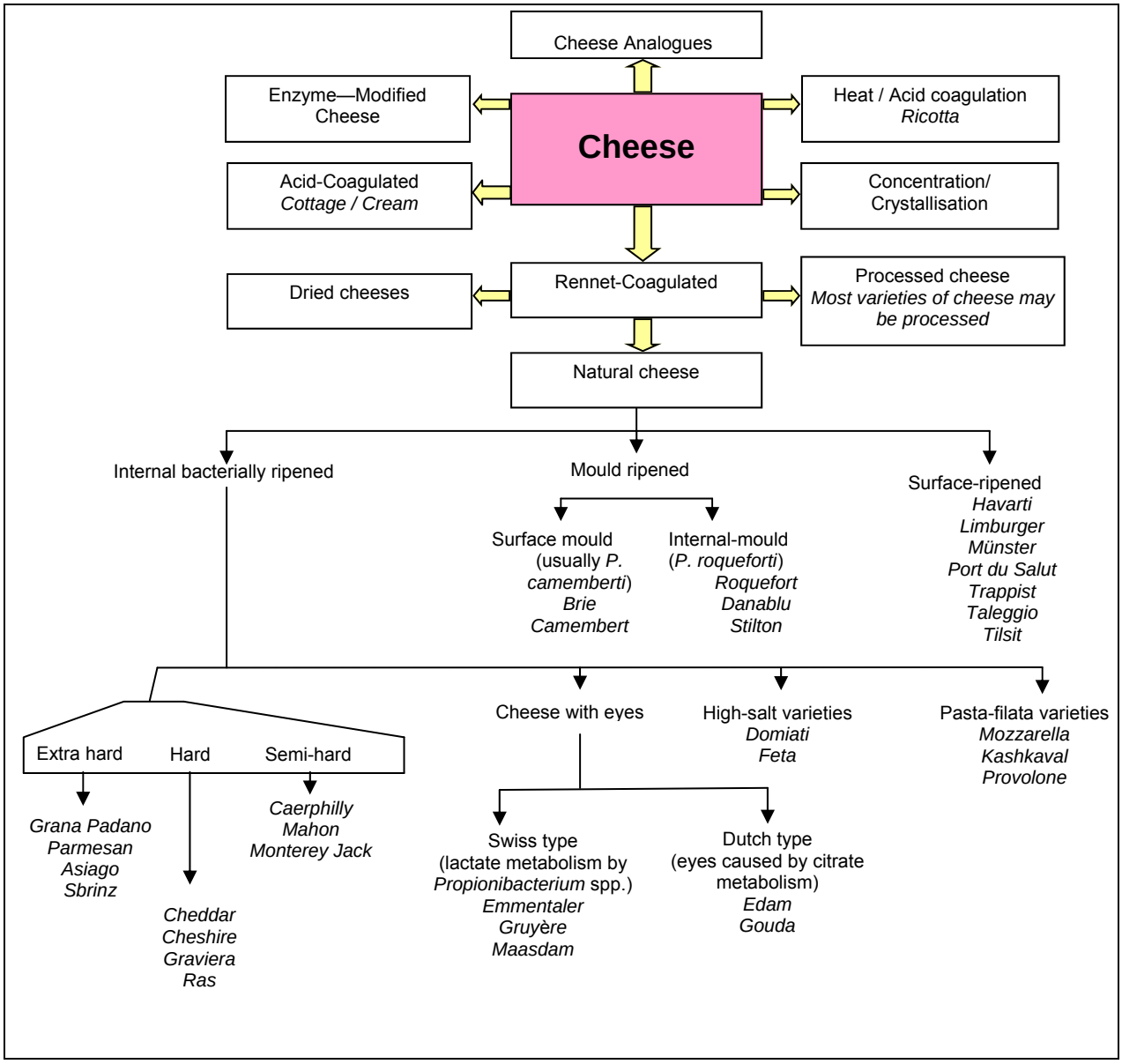

Figure 2: Classification of cheese into super-families (modified from Fox *et al.*, 2000)

Table 5: Principal categories of cheese

Internal Bacterially Ripened	
Extra hard varieties	Extra hard cheeses are characterised by a hard granular texture following ripening for a long period (usually 6 - 24 months). Examples are the Italian "Grana" types, Asiago and "Pecorino" cheeses.
Hard varieties	Hard cheeses are typically milled with dry salting of the curd. Cheddar cheese, originating in England, is one of the most important cheese varieties made worldwide. Other British Territorial hard cheese varieties include Cheshire, Derby, Gloucester and Leicester.
Semi-hard varieties	The description of a cheese as semi-hard is arbitrary and distinction between this and other groups (*e.g.* hard, smear-ripened and pasta-filata) may not be clear. Semi-hard varieties include Colby and Monterey, Lancashire and Bryndza. Stirring Cheddar-type cheese curd inhibits the development of curd structure and results in a cheese with higher moisture content and a softer texture.
Cheese with eyes (Swiss type)	Semi-hard cheeses with propionic acid fermentation include Maasdamer, Emmentaler and Jarlsberg. The propionic acid fermentation produces numerous large openings called "eyes". Characteristics of this category include: formation of eyes and ripening at elevated temperatures.
Cheese with eyes (Dutch type)	Unlike the eye formation using propionic acid formation, Gouda and related type cheese eye formation is through the metabolism of citrate.
Pasta-filata cheeses	These cheeses are semi-hard varieties, also known as kneaded or plastic curd cheeses and include Mozzarella, Provolone and Kasseri. These cheeses are heated to a high temperature, kneaded and stretched.
Cheeses ripened under brine	Feta, Domiati and related species are also referred to as pickled cheeses as they are ripened under brine.
Mould ripened varieties	
Surface mould ripened varieties	Soft cheeses characterised by the growth of *Penicillium camemberti* on the cheese surface are usually high moisture and have relatively short maturation and shelf-life.
Internal mould ripened varieties	Characterised by a network of blue and green veins caused by the growth of *Penicillium roqueforti*. Examples include Cabrales, Gorgonzola, Stilton and Roquefort.
Surface smear ripened varieties	Smear cheeses are characterised by the growth of complex Gram-positive microflora on the surface during ripening. Although most varieties in this group are soft or semi-hard, a surface flora may also develop on hard cheeses such as Gruyère.
Acid-curd cheese	Acid-coagulated cheese is made by acidifying milk to a pH of 4.6 resulting in coagulation. These cheeses are characteristically high in moisture and consumed fresh, however, they may be ripened. Cottage and Quarg varieties are acid-curd cheeses.
Heat/Acid cheese	These cheeses are produced from rennet cheese whey, with a small amount of milk added, as well as the addition of an acidifying agent and exposure to heat (85 - 90°C). The coagulant is then pressed into moulds, packed in ice and allowed to drain. The most common variety is Ricotta.

5. Raw milk cheeses

While cheese has been produced for centuries using raw milk, the introduction of pasteurisation in the 20th century has had an important role in enhancing the safety of many cheeses. While pasteurisation kills pathogens that might be present in raw milk, spores of *Clostridium* spp. and *Bacillus* spp. and thermoduric organisms can survive. Pasteurization also inactivates several enzymes in the milk including lipase and alkaline phosphatase while some enzymes from psychrotrophic bacteria (acid phosphatase and xanthine oxidase) withstand pasteurisation (Grappin and Beuvier, 1997). Heat treatment in excess of High Temperature Short Time (72°C – 15 seconds) pasteurisation may damage the cheesemaking properties of the milk.

A number of studies have compared the levels of microorganisms found in raw milk cheeses and their corresponding pasteurised cheese varieties. Generally raw milk cheeses have a natural, highly variable microflora not found in pasteurised milk cheeses. Studies of various cheese types have indicated higher counts of Streptococci, Lactobacilli, Enterococci and Propionibacteria in raw milk Cheddar, Swiss, Raclette and Castellano cheeses at the end of ripening than the corresponding pasteurised milk cheeses (Beuvier and Buchin, 2004).

Raw milk cheeses are often extolled by cheese connoisseurs as having greater and stronger flavour than cheeses produced with pasteurised milk. Raw milk cheeses tend to ripen faster than cheeses made from milk where the indigenous microflora has been eliminated. This is thought to result in stronger flavour and/or odour development. Significant research has been undertaken to compare ripening in raw milk and pasteurised milk cheeses. In all the cases studied, the contributing factor appears to be directly linked to the activity of indigenous microflora of the milk, rather than inactivation of indigenous enzymes or other heat induced changes (Beuvier and Buchin, 2004).

Artisan or artisanal cheese implies that a cheese is produced primarily by hand, in small batches, with particular attention paid to the tradition of the cheese maker's art, and thus using as little mechanisation as possible in the production of the cheese. Raw milk cheeses are more commonly produced by artisan cheese makers.

6. Consumption of cheese

Food consumption data can be used to determine how and what people eat and is required to assist in the characterisation of the risk from exposure to microbiological hazards in food. Information on the amount of food consumed, the frequency of consumption of the food, and the form in which a food is consumed is used to estimate the likely exposure[14] to a particular hazard from a food.

Food consumption data sets differ depending on how the information is collected and reported, the form of the foods (*i.e.* raw agricultural commodities or foods as consumed), and whether information on consumption by population subgroups is available. There are generally two types of consumption data available. Total population summary data derived from food production statistics, and food consumption surveys which contain detailed information about the types and amounts of foods consumed by individuals or households and sometimes the frequency with which these foods are consumed.

Australian food production statistics and consumption data from the Australian National Nutrition Survey (NNS) for pasteurised cheese has been used to provide an indication of the likely consumption of raw milk cheeses in Australia. Where available, comparative international data from countries where raw milk cheeses are produced has also been used to provide an indication of the amount of raw milk cheese manufactured in comparison to pasteurised milk cheeses.

6.1 Production of raw milk cheese

International data on the production of raw milk cheese is limited, whilst in Australia raw milk cheese is not produced.

Annual production of raw milk cheese in Europe is estimated at approximately 700,000 tonnes; the largest producers being France, Italy and Switzerland. In comparison, it is estimated that in 2004 the total cheese production in the EU was 8,550,000 tonnes (IDF, 2005).

Raw milk cheeses represent approximately 10% of the total cheese production in the EU and Switzerland; however this varies between EU countries. For example, France produces approximately 1,600,000 tonnes of ripened cheeses of which 200,000 tonnes are manufactured from raw milk (15% of French production) (Lortal, 2005), whereas only approximately 5,000 tonnes was produced (1.5% of total cheese production) in Spain during 2001 (Beuvier and Buchin, 2004).

In Switzerland, 59.5% of cheeses produced, including Emmentaler, Gruyère, Tilsiter, and Appenzeller, are made from raw milk. Another 28.7% of cheeses produced are made from either pasteurised or raw milk, whereas soft cheeses and Mozzarella cheese are only made from pasteurised milk (Schaellibaum, 2005).

The production of specific French raw milk cheeses are listed in Table 6.

[14] Exposure is a function of two components (i) the concentration of the pathogen on or in the food of interest and (ii) the amount of food consumed

Table 6: Production of French raw milk cheese (Lortal, 2005)

Cheese type	Production (tonnes)	Cheese	Production (tonnes)
Comte	40,000	Morbier	6,500
Roquefort	18,000	Tomme	5,000
Reblochon	17,000	Beaufort & Abondance	5,000
Camembert	12,500	Saint Nectaire	5,500
Brie	8,500	Goat raw milk cheeses etc	5,100
Cantal	7,000		

In Canada it is estimated that the production of raw milk cheese accounts for 15% of all speciality cheese production (Agriculture and Agri-Food Canada, 2005) and therefore 8.7% of all cheese produced (Agriculture and Agri-Food Canada and Dairy Farmers of Canada, 2005).

There is limited information available on the types of raw milk cheeses produced. A breakdown of worldwide and Australian pasteurised cheese production by cheese type[15,16] (Dairy Australia, 2005) is shown in Figures 3 and 4 respectively.

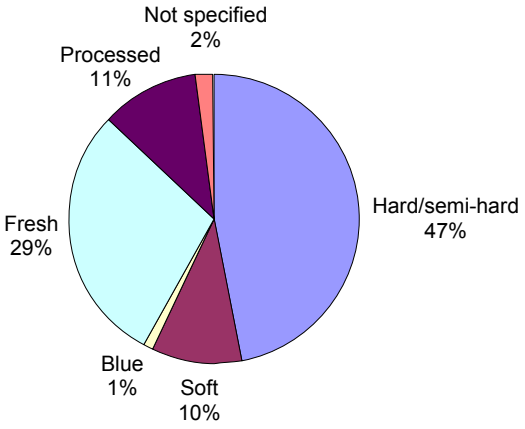

Figure 3: Cheese production world-wide (1999)

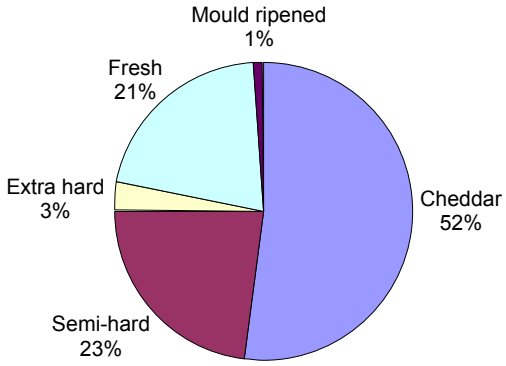

Figure 4: Cheese production in Australia (2004)

[15] Hard and semi-hard cheese – all cheeses except blue mould, blue/white mould and fresh cheeses with moisture content on fat free basis of 67% or less *e.g.* Caerphilly, Cantal, Cheddar, Chesshire, Chester, Danbo, Edam, Emmentaler, Feta, Fontina, Gouda, Grana, Gruyere, Havarti, Jarlsberg, Lancashire, Parmesan, Provolone
Soft cheese – All cheeses, excluding hard and semi-hard, blue mould, blue/white mould, and fresh cheese, *e.g.* Camembert, Hervé, and Italico
Blue cheese – All blue (or green moulded cheeses including blue/white moulded cheeses *e.g.* Danablu, Edelpilz, and Gorgonzola
Fresh cheese – Uncured or unripened cheeses, *e.g.* cottage cheese, Mozzarella, Ricotta and Quark
Processed cheese – cheeses used in catering *e.g.* pizzas, burgers, sandwiches, salads etc

[16] Cheddar - includes other Cheddar types *e.g.* Colby, Cheshire, Gloucester, Lancashire, Leicester, Nimbin
Semi-hard – includes mozzarella, pizza, Edam, Gouda, other eye cheeses (*e.g.* Swiss, Emmenthal, Fontina, Harvarti, Samsoe, Tilsit, Buetten, Vacherin), other semi-hard cheeses (*e.g.* Bakers, Casalinga, Goya)
Extra hard – includes Parmesan, Pecorino, Romano, Pecorino, Melbourno, Pepato, Parmagiano
Fresh – includes Cottage, Cream, Feta, Neufchatel, Ricotta, Quark, Stracchino, Mascarpone
Mould-ripened – includes Blue vein, Brie, Camembert and other mould ripened cheeses

Worldwide[17] hard/semi-hard cheese is the predominant cheese type produced, accounting for approximately 54% of total production. Hard/semi-hard cheese accounts for nearly 100% of total production in some of the largest cheese producing countries such as the Netherlands and New Zealand, and around 90% of production in Sweden, Norway and Switzerland. Hard/semi-hard cheeses account for almost 75% of total cheese production in Australia and over half of all cheese produced is Cheddar.

Soft and blue cheeses accounted for approximately 11% of total cheese production world-wide in 1999. France is the leading country for producing soft and blue cheeses, accounting for nearly half of their total production (IDF, 2001), whereas soft and blue cheese account for only approximately 1% of total cheese production in Australia.

6.2 Consumption of raw milk cheese

As with information pertaining to production of raw milk cheeses, data on the consumption of raw milk cheese is also extremely limited. Although raw milk cheeses are not produced in Australia, a select few raw milk cheeses are currently permitted to be sold (imported) in Australia including: extra hard type cheeses (*e.g.* parmesan types),Emmentaler, Gruyere, Sbrinz and Roquefort cheese. Data on the quantity of cheeses imported was not available. Hence accurate data on the consumption of raw milk cheeses in Australia was also not available.

Nevertheless, where international raw milk cheese production data and cheese consumption data is known, an estimate of annual per capita consumption of raw milk cheeses can be made.

In France the estimated per capita consumption of raw milk cheese is roughly 3.8 kg, based on raw milk cheese production being 15% and per capita consumption of cheese being 25.3 kg/year. Similarly using Canadian consumption and production data, per capita consumption of raw milk cheese is approximately 1.2 kg/year. However, without knowing export and import figures for raw milk cheeses, there is some uncertainty surrounding these estimates.

While there is very limited data on the consumption of raw milk cheeses in Australia, consumption of cheese per capita in Australia is lower (~12 kg) than countries in Europe (~18.9 kg), USA (14kg) and Canada (15.5kg) (IDF, 2005) (Figure 5). This would suggest that even if raw milk cheeses were readily available in Australia, consumption may be lower than in other countries.

[17] EU (except Italy and Portugal), Iceland, Norway, Switzerland, Estonia, Hungary, Poland, Israel, Japan, Australia, New Zealand, Canada, USA, Argentina and Southern Africa – representing 75% of all global production

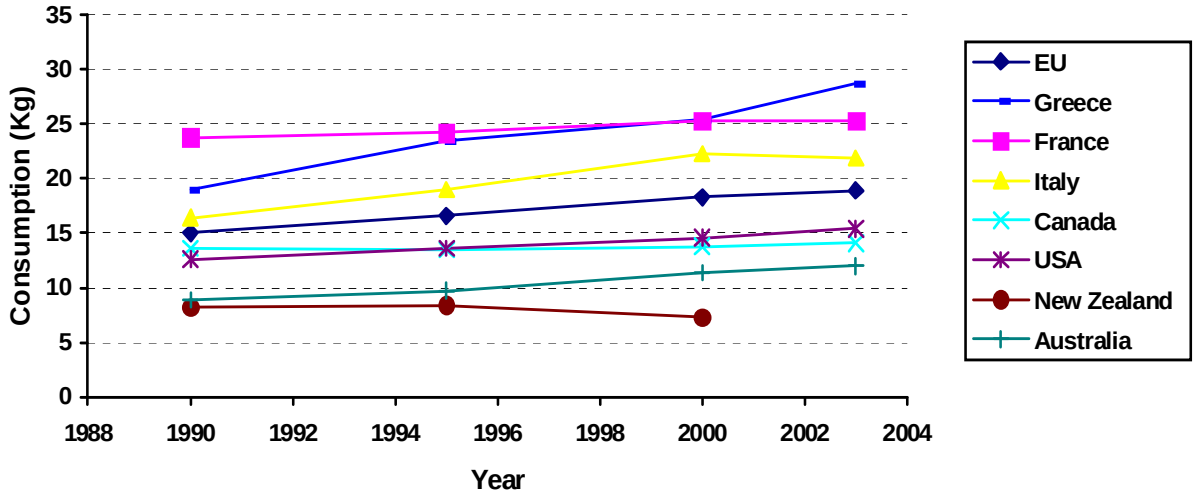

Figure 5: Consumption of cheese per capita 1990-2003 (IDF, 2001; IDF, 2005)

Consumption data derived from Australian food production statistics and from the Australian NNS[18] conducted during the period from February 1995 - March 1996, suggest that hard cheeses are the most commonly consumed cheese in Australia (Tables 7 and 8). In addition, almost all the hard/semi-hard cheese consumed is Cheddar and Cheddar types, although there is an increasing trend from Cheddar to non-Cheddar cheese varieties (Dairy Australia, 2004). It is unlikely that the availability of raw milk cheeses would significantly alter these consumption trends.

Table 7: Australian consumption of cheese per capita 1990-1999 (kg/year) (IDF, 2001)

Cheese Type	1990	1994	1999
Hard/Semi-hard	5.7	5.8	6.9
Soft (including blue)	NA	0.1	0.2
Fresh	0.8	0.8	1.2
Processed	2.4	2.5	2.4
Total	**8.9**	**9.3**	**10.7**

Table 8: Summary of cheese consumption from 1995 Australian NNS (Australian Government Department of Health and Family Services, 1997)

Product	Average no. people surveyed consuming product (%)	Average amount consumed per day (g)*
Extra hard cheese	2.3	8
Swiss-type cheeses	0.3	40
Cheddar	25.6	35
Blue cheese	0.5	37
Feta	0.6	41
Camembert	0.6	35

* The consumption figures listed are for **consumers** of listed cheese only.

[18] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24 hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

Data from the NNS indicates Cheddar (25.6%) was the most consumed cheese with an average of 35 g consumed. Consumption of extra hard, Swiss-type, blue, Feta and Camembert cheeses is significantly lower with only 2.3%, 0.3%, 0.5%, 0.6% and 0.6% of the population surveyed consuming these cheese types, respectively. Consumption of these cheese varieties was very low which would suggest the potential consumption of raw milk cheeses may also be very low.

6.3 Summary

The production of raw milk cheese worldwide is relatively low, 0.7 million tonnes compared to total cheese production of 8.55 million tonnes in 2004 in the EU. Even for countries where raw milk cheeses are traditionally consumed (*e.g.* France) only 15% of all cheeses produced are made from raw milk. Worldwide, hard/semi-hard cheese represents approximately 54% of all cheese production, whilst in Australia they account for approximately 75% of production with Cheddar cheese the principal variety.

Per capita consumption data indicate that consumption of raw milk cheeses is also relatively low. High cheese consuming countries such as France consume around 3.8 kg (15%) of raw milk cheese per year compared to total cheese consumption of 25.3 kg. Australian annual per capita cheese consumption is significantly lower at around 12 kg per year and the potential consumption of raw milk cheese would also be considerably lower.

It cannot be assumed that the same proportion of the population who currently consume cheese would also consume raw milk cheese. Furthermore, consumption of extra hard, Swiss-type, blue, Feta and Camembert cheeses is already very low which indicates the potential consumption of these cheese varieties, if made from raw milk, would also be extremely low. It is also likely that those who would consume raw milk cheese will not increase their overall cheese consumption, but instead substitute consumption of pasteurised cheese with raw milk cheese.

7. Foodborne illness associated with raw milk cheeses

7.1 Foodborne illness

Prior to the introduction of pasteurisation, dairy products were frequently implicated in foodborne illness. Foodborne illness arising from the consumption of contaminated cheese has been documented from as early as 1884 (Zottola and Smith, 1991).

During the first half of the 20th century typhoid was responsible for many outbreaks of foodborne illness in the US (926 cases and 18 deaths) due to the consumption of contaminated cheese (mainly Cheddar cheese). Six outbreaks over a period of 40 years were due to *Salmonella* spp. (Table 9). *Staphylococcus* spp. and *Streptococcus* spp. were also associated with outbreaks involving cheese.

Table 9: US outbreaks – 1917 1944 (adapted from Zottola and Smith, 1991)

Year	Organism	Cases (deaths)	Cheese type
1917	*Salmonella typhi*	64 (4)	Cheddar
1925	*Salmonella typhi*	29	Cheddar
1941	*Salmonella typhi*	23 (1)	Cheddar curd
1944	*Salmonella typhi*	80	Romano-Dolce
1944	*Salmonella typhi*	246 (13)	Green Cheddar
1945	*Salmonella* spp.	484	Colby
1917-1944	*Staphylococcus* spp. and *Streptococcus* spp.	265	Asiago, Colby, NY Herkimer, Cheddar, Cottage and imported Albanian cheese

In Canada during the period 1932 - 1939, six epidemics of typhoid involving 760 cases and 71 deaths were reported. One outbreak in 1939 involving 100 cases and 11 deaths from typhoid were reportedly due to Cheddar cheese. Forty cases and 6 deaths of typhoid were reported in 1943, and were attributed to Cheddar cheese. Again in 1944, Cheddar cheese was implicated in 83 cases of foodborne illness and 7 deaths.

The US passed regulations in 1949 (21CFR133) requiring milk used for cheesemaking to be pasteurised, or cheese made from raw milk to be held for at least 60 days at temperatures of not less than 35°F (1.7˚C).

A review by Johnson *et al.* (1990a) of foodborne illness transmitted via cheese produced in the US identified only six outbreaks during the period 1948 - 1988. Of these outbreaks, post pasteurisation contamination, improper pasteurisation and milk quality were cited as the most common causative factors.

Foodborne illness associated with the consumption of cheese (pasteurised, raw milk and unknown heat treatment) produced from cow, goat and sheep milk during the period 1973 - 2006 are summarised in Table 10. Outbreaks by milk origin (cow, goat and sheep) are detailed in Appendix 4.

Table 10: Global outbreaks attributed to cheese - 1973 2006 (Appendix 4)

Organism	Raw	Pasteurised	Unknown	Total
Salmonella spp.	17 (14 cow, 3 goat)	11 (10 cow, 1 goat)	2 (cow)	**30**
Escherichia coli	8 (5 cow, 3 goat)	-	1 (cow)	**9**
Staphylococcus aureus	5 (3 cow, 2 sheep)	2 (cow)	1 (cow)	**8**
Listeria monocytogenes	9 (8 cow, 1 goat)	2 (cow)	2 (cow)	**13**
Brucella melitensis	11 (5 cow, 6 goat)	-	-	**11**
Campylobacter spp.	1 (sheep)	-	-	**1**
Streptococcus	2 (1 cow, 1 goat)	-	-	**2**
Mycobacterium bovis	1 (cow)	-	-	**1**
Clostridium spp.	-	2 (cow)	-	**2**
*Coxiella burnetii**	2 (goat)	-	-	**2**
Shigella spp.**	1 (sheep)	1 (cow)	-	**2**
Unknown	1 (cow)	2 (cow)	-	**3**
Total	**58**	**20**	**6**	**84**

* may be attributed to workers and not cheese
** may be attributed to a dairy worker

A total of 84 reported outbreaks have been attributed to the consumption of cheese during the period 1973 - 2006. Of these, 69% (58/84) were from raw milk cheeses, 23.8% (20/84) were attributed to cheese made from pasteurised milk and 7.2% (6/84) were associated with cheese where the heat treatment and/or source of milk were unknown.

A comparison of outbreaks attributed to cheeses made from both pasteurised and raw milk is shown in Figure 6. *Salmonella* spp. accounted for the highest number of outbreaks, with cheeses made from raw and pasteurised milk accounting for 29% (17/58) and 60% (11/20), respectively. *Listeria monocytogenes* accounted for 15% (9/58) and 10% (2/20) of raw and pasteurised milk cheese outbreaks. *Brucella melitensis* and *E. coli* were responsible for 19% (11/58) and 14% (8/58) of outbreaks associated with cheeses made from raw milk respectively, whereas no outbreaks were reported for either *Brucella* spp. or *E. coli* from cheeses made from pasteurised milk. Nearly all of the outbreaks attributed to cheeses contaminated with *Brucella* spp. were Mexican-style[19] cheese. *S. aureus* accounted for 9% (5/58) and 10% (2/20) of outbreaks attributed to cheeses made from raw and pasteurised milks respectively.

Campylobacter spp., *Streptococcus* spp., *M. bovis* and *Coxiella* spp. were only associated with outbreaks implicating raw milk cheese, whilst *Clostridium* spp. were associated with cheese from pasteurised milk only. Outbreaks associated with *Shigella* and of unknown aetiology were associated with both raw and pasteurised cheese.

An analysis of all outbreaks by region is shown in Table 11. Europe accounted for the highest number of cheese related outbreaks 54.7% (46/84), followed by the USA 30.9% (26/84). Where outbreaks were attributed to cheese made from raw milk, Europe accounted for the highest number of outbreaks 62% (36/58) followed by the USA 22.4% (13/58).

[19] Mexican style cheeses include: Queso blanco, Quesco Fresco, Panela Ranchero and soft Hispanic cheese. These cheeses do not have a standard of identity and most are rennet coagulated, with or without lactic starter culture and may contain added organic acids (Fox *et al.*, 2004).

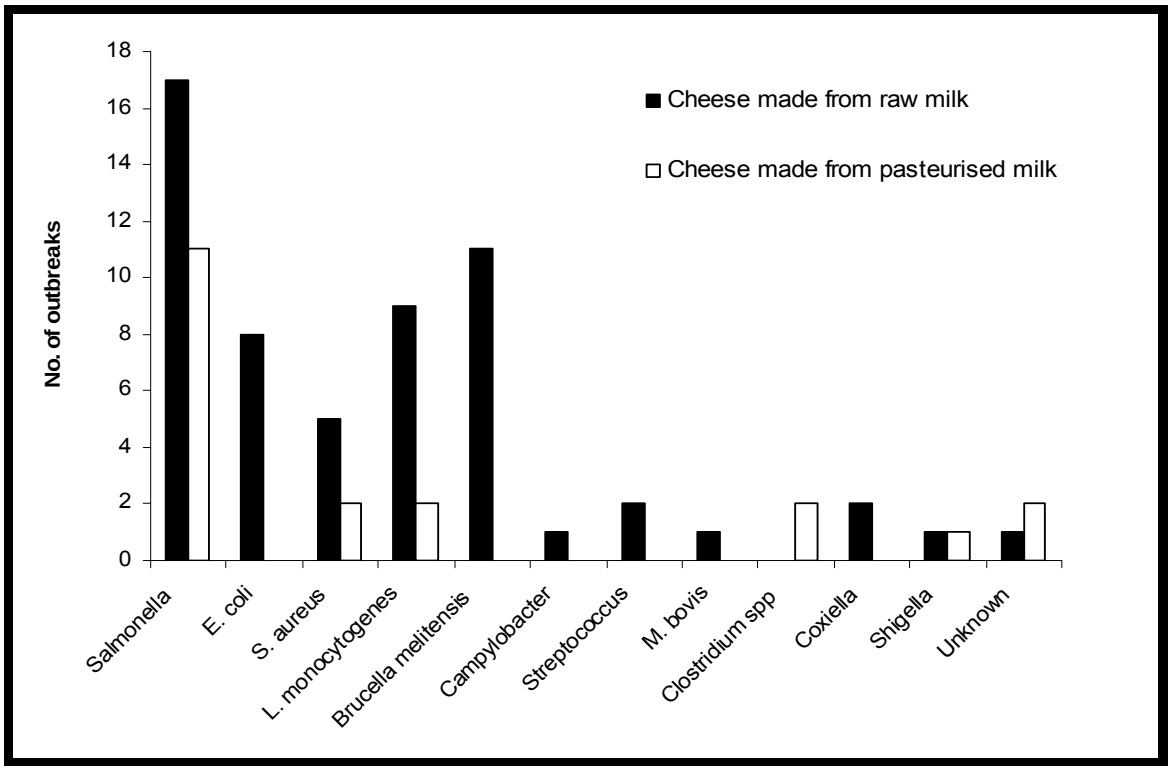

Figure 6: Raw and pasteurised milk cheese outbreaks by aetiology (1973 -2 006)

Table 11: Outbreaks attributed to consumption of cheese by region (1973 - 2006)

Region	Raw	Pasteurised	Unknown	TOTAL
USA	13 (11 cow, 2 goat)	12	1	26
Canada	6 (5 cow, 1 goat)	2	1	9
Europe	36 (21 cow, 11 goat, 4 sheep)	6	4	46
Other	3 (2 cow, 1 goat)	-	-	3
TOTAL	**58**	**20**	**6**	**84**

An analysis of raw milk cheese mediated outbreaks by cheese type implicates soft cheese as the most common transmission vehicle (Table 12). Soft and Mexican-style cheeses accounted for 27.6% (16/58) and 15.5% (9/58) of all reported raw milk cheese outbreaks, respectively. Goat cheese (including soft, fresh and Mexican-style) was responsible for 27.6% (16/58) of raw milk cheese outbreaks.

Table 12: Outbreaks attributed to raw milk cheese by cheese-type (1973-2006)

Cheese type	No. of outbreaks	Cheese type	No. of outbreaks
Cheddar/gouda	3	Mexican	9
Blue	1	Goats	16
Soft	16	Sheep	4
Fresh	2	Unknown	7

7.2 Summary

Even with the advent of pasteurisation in the 20th century, cheese and in particular raw milk cheeses continue to be associated with outbreaks of foodborne illness. Outbreaks attributed to raw milk cheeses account for 70% of all cheese outbreaks, however, raw milk cheese only represents about 10% of all cheese sold.

Contamination of raw milk cheese with *Salmonella* spp., *Brucella* spp., *L. monocytogenes*, and *E. coli* accounted for the majority of reported outbreaks. Cheeses most commonly implicated are typically cheeses with high moisture content and little or no maturation or ripening.

Caution should be exercised when analysing and interpreting epidemiological data as surveillance systems vary widely and outbreaks of foodborne illness are significantly under-reported. Sporadic cases are rarely investigated and sources of illness for these cases are very difficult to establish. Estimates of foodborne illness are derived from available data and only provide a 'snapshot' of the incidence of foodborne illness attributed to raw milk cheese.

During the period 2006 - 2008, further outbreaks of foodborne illness involving cheese have continued to occur in various countries. As recently as September 2008, a major outbreak in Canada involving retail contamination of cheese with *L. monocytogenes* caused over 14 illnesses and at least one death. Three cases of listeriosis resulting in two miscarriages were also linked to consumption of soft cheeses in North Carolina in 2007. Also in 2007, the Quebec Ministry of Agriculture linked a case of listeriosis to a raw goat milk cheese from La Ferme écologique coop d'Ulverton.

At the same time a number of food recalls have been initiated due to the presence of pathogens in raw milk cheeses. For example, in September 2008 the Food Safety Authority of Ireland advised of the withdrawal of four French soft cheeses due to the presence of *L. monocytogenes*.

8. Occurrence of pathogens in raw milk cheeses

Raw milk cheese has been shown to contain a variety of pathogens including, but not limited to: *Brucella* spp., *Campylobacter* spp., pathogenic *E. coli, L. monocytogenes*, *Salmonella* spp. and *S. aureus.* Table 13 provides a brief summary of these microorganisms, the severity of associated illness and the availability of epidemiological data.

There is no data on the presence or absence of pathogens in raw milk cheeses in Australia, as raw milk cheeses are not manufactured domestically.

Data from international studies and the EU Rapid Alert System (European Food Safety Authority, 2006) provides evidence of the periodic occurrence of pathogens in raw milk cheeses (Appendix 5).

Table 13: Summary of identified microbiological hazards associated with raw milk cheeses

Organism	Shed directly in milk[#]	Contaminant of raw milk[##]	Severity of illness[§]	Cheese products implicated in foodborne illness
Brucella spp.	Yes	Yes	Serious	++
Campylobacter jejuni/coli	Yes	Yes	Serious^	+
Enterohaemorrhagic *E. coli*	Yes	Yes	Serious	++
Listeria monocytogenes	Yes	Yes	Severe^	++
Salmonella spp.	Yes	Yes	Serious^	++
*Staphylococcus aureus***	Yes	Yes	Mild	++

[#] Transmission via udder, mastitis, etc
[##] Transmission via faeces, environment, etc
[§] Qualitative Framework (Appendix 1)
^ Susceptible populations
\+ Reported - but rare
++ More commonly associated with illness
** enterotoxin is heat stable

While a range of pathogens associated with raw milk cheeses have been identified in the literature, this risk assessment only considers a selection of pathogens including: *Campylobacter* spp.*, S. aureus, L. monocytogenes, E. coli* (EHEC), and *Salmonella* spp. The microorganisms selected for consideration are representative of those pathogens that have been clearly implicated in foodborne illness and may be present in raw milk, either directly transmitted via the mammary gland or via faecal or environmental contamination.

Brucella spp. has been responsible for a number outbreaks of foodborne illness caused by the consumption of raw milk cheeses and its survival in many cheese varieties is well documented (Ryser, 2001). *B. abortus* has reported survived in Emmental, Gruyere, Tilsit, Cheddar, Camembert and pecorino cheeses.

Mycobacterium bovis has also been implicated in recent outbreaks in fresh/soft raw milk cheesesand has been shown to survive in various cheeses including Camembert, Cheddar and Tilsit (Ryser, 2001).

Tuberculosis resulting from milkborne transmission of *Mycobacterium bovis* has been drastically reduced in recent times worldwide by a combination of changing milk consumption habits, mandatory pasteurisation and cattle immunization programs (Ryser, 2001).

Until recently, bovine brucellosis was present throughout the world. However, a number of countries have now succeeded in eradicating this disease. These include: Australia, Canada, Israel, Japan, Austria, Switzerland, Denmark, Finland, Norway, Sweden and New Zealand. However, *B. melitensis* remains endemic in southern Europe, west and central Asia, Mexico, South America and Africa.

Brucella abortus in milk producing animals in Australia has been eradicated since 1989 and *B. melitensis* has never been detected in Australian herds. Australia has been recognised as bovine tuberculosis (*M. bovis*) free since 31 December 1997, and continues to conduct screening programs to monitor any *M. bovis* infection in dairy cattle.

The Australian Quarantine and Inspection Service and Biosecurity Australia maintain import requirements focussed on animal health and biosecurity issues. Import conditions are currently being reviewed by Biosecurity Australia for Dairy Products, which includes consideration of *Brucella* spp. and *Mycobacterium bovis*. It should be highlighted that should these organisms be introduced into Australia through importation of contaminated raw milk products, they would pose a risk to individuals from consumption of these products.

Mycobacterium bovis and *Brucella* spp. have not been specifically considered in this assessment.

8.1 Raw milk cheese – cow origin

Surveys show that various pathogens including *E. coli* (EHEC), *L. monocytogenes*, *Salmonella* spp. and *S. aureus* have been isolated from raw cow milk cheeses (Appendix 5: Tables 1 - 6). Pathogens were detected in 53 of 86 (62%) studies identified in the literature. *Campylobacter* spp. have rarely been detected in raw cow milk cheese. In one study in Switzerland, however, 6.5% of samples tested positive using PCR (93 samples) (Appendix 5: Table 2).

E. coli has been tested for in many countries and across a variety of raw milk cheese types. *E. coli* (generic and pathogenic) was detected in 17 of 28 (61%) identified studies examining raw milk cheese in the literature. Prevalence of *E. coli* has been reported between 0 - 81% (Appendix 5: Table 3) and at levels up to 3.2×10^6 cfu/g (Appendix 5: Table 13). *E. coli* O157 has been detected at a prevalence of up to 5.6% in Belgium fungal ripened soft cheese, while verocytotoxin producing *E. coli* (VTEC) and STEC have been detected in 1.5% of German soft and semi-soft cheese and between 5.2 - 30.5% of French soft cheese (Appendix 5: Table 3). Contamination levels of *E. coli* O157 have been recorded in raw milk cheese at levels up to 6×10^4 cfu/g (Appendix 5: Table 13).

L. monocytogenes was detected in 17 of 21 (81%) identified studies examining raw milk cheese, with contamination rates ranging between 0 - 65% (Appendix 5: Table 4). Levels of *L. monocytogenes* have been reported up to 1×10^5 cfu/g in soft and semi-soft cheese (Appendix 5: Table 17).

Salmonella spp. were detected in 6 of 16 (38%) identified studies examining raw milk cheese in the literature. Isolation of *Salmonella* spp., whilst frequently not detected in surveys of raw milk cheese in international studies, have occurred from raw milk semi-hard cheese in Turkey at 2.4% and raw milk unripened Van otlu Turkish cheese at 6% (Appendix 5: Table 5).

S. Typhimurium and *S.* Heidelberg have been reported in raw milk Cheddar cheese at 9.3 cells/100g and 1.8 cells/100g, respectively (Appendix 5: Table 16).

S. aureus was detected in 9 of 11 (82%) identified studies examining raw milk cheese in the literature. International data highlights prevalence for *S. aureus* can be up to 100% in raw milk cheese (Appendix 5: Table 6) with levels up to 1.41×10^7 being reported (Appendix 5: Table 14).

8.2 Raw milk cheese – goat origin

There is very little information available in the literature on the prevalence of pathogens in raw milk cheeses made from goat milk. Pathogens were detected in 3 of 4 (75%) identified studies examining raw milk cheese in the literature. Pathogens detected in raw milk goat cheeses include *Brucella* spp., *Salmonella* spp., *E. coli* and *S. aureus*.

Brucella spp. were reported at a prevalence of 46% in raw milk goat cheese in Italy (Appendix 5: Table 7), while a study conducted in Spain returned 0% prevalence in raw milk cheese for *Salmonella* spp. (Appendix 5: Table 8). *E. coli* has been detected in Caprino d'Aspromonte cheese in Italy at levels up to 1×10^8 cfu/g and *S. aureus* has been detected in raw goat milk cheese in Spain at levels of up to 8.8×10^3 cfu/g (Appendix 5: Table 18).

8.3 Raw milk cheese – sheep origin

There is very little information available in the literature on the prevalence of pathogens in raw sheep milk cheese. Pathogens were detected in 5 of 6 (83%) identified studies examining raw milk cheese in the literature. Pathogens detected include *Brucella* spp., *E. coli* and *L. monocytogenes*.

International data suggests that the prevalence of *Brucella* spp. and *E. coli* O157 ranges from 14.2 - 46% and 0 – 3.6%, respectively (Appendix 5: Table 9 and Table 10). A Portuguese study examined 63 samples of soft raw milk cheese and reported a 75% prevalence of *Listeria* spp., 29% prevalence of *L. innocua* and 46% prevalence of *L. monocytogenes* (Appendix 5: Table 11). Schoder et al, (2003) reports levels of *L. monocytogenes* in soft cheese of 2×10^2 cfu/g (Appendix 5: Table 19).

8.4 EU Rapid Alert System

The Rapid Alert System is primarily a tool for exchange of information between authorities in the Member States in the EU in cases where a risk to human health has been identified and measures have been taken, such as withholding, recalling, seizure or rejection of the products concerned.

Examination of rapid alert data between 2003 - 2006 identified 76 notifications associated with cheese. Ten notifications were identified for raw milk cheese and two for pasteurised milk cheese. It was not possible to determine the origin of the remaining 64 cheeses (Appendix 5: Table 20). Nevertheless, the data does highlight the range and frequency of microbiological hazards that may be associated with various cheeses.

Contamination of cheese with *L. monocytogenes* accounted for 68% (52/76) of all cheese alerts and 60% (6/10) of those where raw milk cheeses could be identified. *E. coli*,

Salmonella spp. and *S. aureus* accounted for 12% (9/76), 9% (7/76), and 4% (3/76) of all cheese alerts, respectively.

Gorgonzola was responsible for 26% (16/76) of all cheese alerts where cheese types could be identified, soft cheeses were responsible for 22% (14/76), and goat cheese was responsible for 11% (7/76) (Appendix 5: Table 21).

Of all reported rapid alerts, *L. monocytogenes* was detected in nearly all cheese types. Detections of *Salmonella* spp. were found in Roquefort (1), Camembert (2), Fresh (1), goat (2) and ricotta (1) cheeses. *E. coli* was detected in cheese curd (2), hard cheese (1), soft cheese (1), white mould cheese (1), unidentified raw milk (1) and pasteurised (1) cheese. *S. aureus* was detected in an unidentified raw milk cheese, an artisan and a parmesan cheese (Appendix 5: Table 21).

8.5 Summary

There is little published information available on the incidence and prevalence of pathogens in raw milk cheese. International microbiological survey and monitoring data demonstrate that pathogens can be isolated from raw milk cheeses. Pathogens detected in raw milk cheeses internationally have included *Brucella* spp., *Mycobacterium bovis*, *Salmonella* spp., pathogenic *E. coli*, *L. monocytogenes* and *S. aureus*.

Particular mention should be made about the prevalence of *Brucella* spp. and *M. bovis* internationally. Although *Brucella* spp. and *M. bovis* have been isolated from raw milk cheese it is important to note that Australia has been free from *B. abortus* since 1989 and *B. melitensis* has never been reported in Australian livestock. In addition, Australia has been recognised as bovine tuberculosis (*M. bovis*) free since 31 December 1997. However, the contamination of *Brucella* spp. or *Mycobacterium bovis* in imported products would pose a risk in raw milk cheeses as their survival in these products has been documented (Australian Quarantine and Inspection Service, 1999).

9. Manufacture and safety of raw milk cheese

The process of making cheese is similar whether produced from pasteurised or raw milk. The primary difference being that milk used for the production of raw milk cheese does not undergo an initial pathogen elimination step such as pasteurisation or thermisation during milk pre-treatment.

The production of cheese varieties, regardless of milk source (cow, sheep or goat milk) generally follows a similar process, as illustrated in Figure 7.

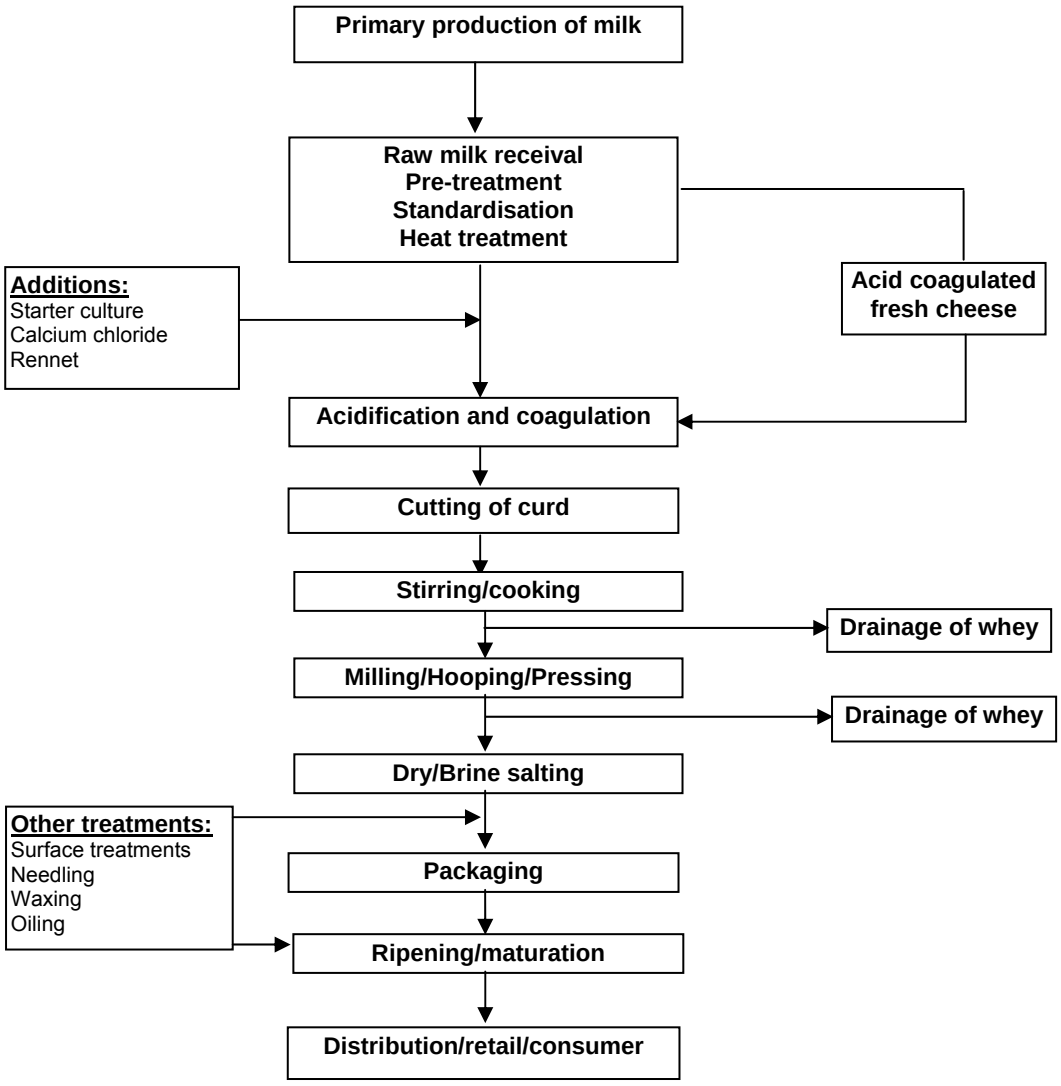

Figure 7: Overview of major steps in the manufacture of cheese

Cheese production involves initial formation of a coagulum in which milk proteins (caseins) are clotted, entrapping milk fat, water and other water soluble components. The coagulum is produced through a process of coagulation and acidification. This can be accomplished either through use of native microflora in raw milk, starter cultures, heat, acid and rennet or a combination of these. The most common way to produce a coagulum is through the addition of starter culture and rennet. The coagulum is then cut (forming curds) and may be cooked and/or stirred, resulting in separation of the whey, which is then drained from the curds. Curds may then be salted before being pressed into moulds or by immersion in a brine solution after pressing. Unripened cheese is then stored under controlled time and temperature conditions to mature.

While the general cheesemaking steps are fairly standard, steps vary depending upon the type of cheese being made. Some cheeses are manufactured without the use of rennet, while others are acidified only by the addition of acid[20]. The use of various starter cultures and adjunct cultures impart different physical and organoleptic properties on the cheese. Calcium plays a fundamental role in the clotting process for rennet set cheeses and enhances cheese yield. If calcium levels in the milk are deficient, exogenous calcium chloride solution may be added. In some fresh cheeses, the curds are not heated, which results in less expulsion of whey and a final product with a higher moisture content. Cheese curd may be washed with water, dry or brine salted, stretched, pressed or unpressed. Moisture content will be influenced by the firmness of the coagulum when cut, the size of the curd pieces, any cooking process, the pH, salt concentration, amount of pressing and ripening conditions. The addition of mould spores is required for surface ripened cheeses such as Brie and Camembert, while spores are incorporated in the curd for interior and surface ripened blue cheeses. During the maturation period, a range of conditions are used in ripening rooms, with particular attention on temperatures and times.

While the general principles of cheesemaking are common to most varieties of cheeses, no two batches of the same variety or probably no two cheeses are identical (Fox *et al*., 2004). The cheese environment is a dynamic and complex entity and the cheesemaking process is controlled by a series of interrelated chemical and physical changes (Marth and Steele, 2001).

Cheese is described by Codex Alimentarius in its General Standard for Cheese[21] as being the "ripened or unripened soft or semi-hard, hard and extra hard product, which may be coated, and in which the whey protein/casein ratio does not exceed that of milk, obtained by:

(a) coagulating wholly or partly the protein of milk, skimmed milk, partly skimmed milk, cream, whey cream or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation; and/or
(b) processing techniques involving coagulation of the protein of milk and/or products obtained from milk which give an end-product with similar physical, chemical and organoleptic characteristics as the product defined under (a)".

Codex Standards for various cheeses are outlined in Appendix 6.

[20] Direct acid set cheeses use organic acids, mineral acids or acidogens.
[21] CODEX STAN A-6-1798, Rev.1-1999, Amended 2003

9.1 Cheesemaking factors affecting safety

The processs for making cheese is a complex interplay between various physical, biochemical and biological processes. Intentionally added microorganisms (*e.g.* starter cultures, adjunct cultures etc) as well asundesirable organisms (*e.g.* illness and defect-causing) often require similar conditions (*e.g*. pH, moisture, salt, acidity/type of acid, redox potential, nutrient availability, competition, temperature and anaerobic/aerobic conditions) for growth, survival or inactivation. The cheese environment is dynamic and the cheesemaking process will at different stages be bacteriocidal, bacteriostatic, or conducive to the growth of different microorganisms.

Following milking and cooling of raw milk, several factors can act as hurdles[22] during the cheesemaking process and these influence the growth, survival and/or inactivation of pathogens in cheese. These hurdles include: pre-treatment of raw milk (*i.e.* thermisation, bactofugation, microfiltration, etc); the amount and duration of heat applied at various stages throughout manufacture; the rate and extent of acidification by the starter culture; salt levels; reduced water availability resulting from salting and ripening/maturation, production of bacteriocins by starter cultures; and the effect of selected food additives used in cheesemaking.

The steps in cheesemaking and any associated effect on pathogens are discussed in the following sections.

9.1.1 Microbiological quality of raw milk

The microbiological quality of raw milk plays an integral part in the safety of raw milk cheese as various pathogenic microorganisms may be associated with raw milk. Published international surveys of raw cow milk have detected a range of pathogens including *Brucella* spp., *Campylobacter* spp., *Coxiella burnetii*, pathogenic *E. coli*, *L. monocytogenes*, *Salmonella* spp. and *S. aureus*. Raw milk from goat and sheep sources have been shown to contain *Brucella* spp., *Campylobacter* spp., *C. burnetii*, pathogenic *E. coli, L. monocytogenes* and *S. aureus* (Appendix 7).

Pathogenic microorganisms may be shed directly into the milk via the udder by a diseased[23] or infected[24] animal or may enter milk from the external surfaces of the animals, the environment, the milking environment, equipment or from personnel.

The factors which impact on these routes of contamination include:

- Animal-related factors *e.g.* animal health, herd size, age and production status
- Environment-related factors *e.g.* housing, faeces, feed, soil, and water
- Milking and operation of milking equipment factors

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22 The ***hurdle concept*** (Leistner and Rodel, 1976) describes the effect of multiple factors (*e.g.* temperature, pH, water activity) on microorganisms. Several different hurdles at sub-optimal levels can be used to control the growth of microorganisms in food products, rather than a single, severe hurdle

23 Disease is defined in the OIE Terrestrial Animal Health Code (2007) as the clinical and/or pathological manifestation of infection (http://www.oie.int/eng/normes/mcode/en_chapitre_1.1.1.htm)

24 Infection is defined in the OIE Terristrial Animal Health Code (2007) as the presence of the pathogenic agent in the host (http://www.oie.int/eng/normes/mcode/en_chapitre_1.1.1.htm)

Comprehensive reviews have been undertaken by FSANZ on raw cow and goat milk which identify and discuss the primary production risk factors, routes of contamination and microbiological quality of raw milk (FSANZ, 2006)[25]. Table 14 summarises the key primary production factors affecting the microbiological quality of raw milk. The primary production risk factors associated with raw sheep milk have not been assessed but are likely to closely resemble those identified for raw cow and goat milk.

Table 14: Key primary production risk factors for raw cow and goat milk

Risk factor	Impact on milk safety	Means of controlling the risk
Disease	Diseased milking animals will show increased shedding of pathogens directly into raw milk or faeces which may contaminate the production and milking environment. Infected animals with no signs of disease (asymptomatic carriers) may also harbour and shed pathogens, often intermittently, into milk and faeces.	Animal health (including mastitis) control programs.
Housing and husbandry	Intensive housing practices may increase the risk of contamination of udders due to high stocking density, concentration of waste, stress and soiled bedding.	Good herd management practices. Attention to animal welfare.
Faeces	Faeces may contaminate the exterior of the udder and introduce pathogens into raw milk.	Reduce scouring. Udder hygiene at milking.
Feed	Contaminated or poorly prepared feed may increase faecal shedding of pathogens. Poor nutritional practices will affect scouring.	Control over preparation, storage and distribution of feed, especially silage.
Water	Contaminated water used for stock drinking, teat washing and cleaning increases risk of environmental contamination.	Ensuring water quality is suitable for purpose.
Milking	Poor milking practices, including dirty, chapped or cracked teats, inadequate cleaning and maintenance of milking equipment, and poor personnel hygiene can lead to contamination of raw milk.	Pre and post milking udder emollients/antiseptics. Effective equipment maintenance, sanitation and cleaning practices.
Storage	Inappropriate temperature control of raw milk after milking can lead to growth of pathogens.	Rapid cooling and holding of milk.
Packaging/ Transport	Packaging and poor hygiene may contribute to cross-contamination of raw milk. Inappropriate temperature control of milk during delivery can lead to proliferation of pathogens.	Correct sanitising and packaging procedures. Effective cold chain management.

The frequency of contamination of raw cow, goat and sheep milk with specific pathogens is summarised in Table 15. The data demonstrates the significant variability in prevalence that is observed in different raw milks. A detailed description of this data is contained within Appendix 7.

Table 15: Summary of prevalence data for raw milk contamination (Appendix 7)

Organism	Raw milk contamination		
	Cow	Goat	Sheep
C. jejuni	Australian data: ND International data: ND – 40%	Australian data: 1.39% International data: ND – 0.04%	International data: ND
S. aureus	Australian data: 22.9% (CP Staph) International data: 9.7 – 100%	Australian data: up to 23.3% International data: ND – 96.2%	International data: 7 – 33.3%
L. monocytogenes	Australian data: ND International data: 1 – 60%	Australian data: ND - 6.8 % International data: ND – 5.8%	International data (Found in ewe's raw milk cheese 46%)
E. coli (EHEC)	Australian data: 1 – 3 % International data: ND – 33.5%	Australian data: 7.37% (*E. coli*) International data: ND – 16.3%	International data: ND – 12.7%
Salmonella spp.	Australian data: 6.2% International data: ND – 11.8%	Australian data: 0.2 % International data: ND	International data: ND

ND Not detected

25 Risk Profile of Dairy Products in Australia and Microbiological Risk Assessment of Raw Goat Milk, respectively

9.1.2 Pre-treatment of raw milk

Inappropriate temperature control of milk after milking can lead to the growth of pathogens, either on farm or at the cheesemaking plant.

Raw milk may be subjected to a range of processing operations before being manufactured into cheese. Typical processes include antibiotic testing (if detected, milk is discarded); filtering (removes extraneous matter including large clumps of bacteria); standardisation or formulation of milk, which may include: separation steps such as filtration, centrifugation, and sometimes clarification and homogenisation. Heat treatments such as thermisation[26] and pasteurisation[27] are applied to milk used for pasteurised milk cheese; however milk used in the production of raw milk cheese, does not receive any initial heat treatment prior to cheesemaking.

9.1.3 Initial cheesemaking phase

The initial phase of cheesemaking, sometimes referred to as the manufacturing stages, refers to the acidification, coagulation, cutting and curd cooking, wheying off and salting of the cheese curd prior to ripening.

The temperature of raw milk at the time the starter culture is added is determined by the type of cheese to be made, type of starter, and the desired temperature at the time of coagulant addition, but is generally between 31 - 34^{o}C (Johnson, 2001)

9.1.3.1 Acidification

In order to make most forms of cheese, milk is acidified through the activity of a select group of lactic acid bacteria called the starter culture (which ferment lactose into lactic acid). The choice of starter depends on the desired rate and extent of acid development during manufacture: proteolytic activity, flavour and/or gas formation and other conditions including pH, acidity, salt and temperature profiles. Some cheeses such as direct-set cottage cheese are manufactured by the direct addition of an acid (*i.e*. lactic acid) instead of through use of starter cultures.

Milk has a natural pH of around 6.6, which starts to decrease once the starter culture is added and begins to grow. If the starter culture (usually added at 10^6-10^7 cfu/ml) immediately dominates the native microflora of the milk, the chance of an increase in the population of pathogenic microorganisms in the milk is substantially reduced (Fox *et al.,* 2000). This is due to the dominance of the starter culture in utilising the available nutrients in milk, and the inhibitory effect of declining pH and increasing levels of organic acids.

The viability and fecundity of the starter culture is important as starter culture failure may result in pathogenic and spoilage microorganisms dominating the microbial population

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[26] Requirements for cheeses made in Australia from thermised milk include heating of the milk to 62°C for no less than 15 seconds. An evaluation of the Thermisation requirement in the Code is included in Appendix 15.

[27] Cheese in Australia is made from milk that is pasteurised using a milk heat treatment of 72°C for 15 seconds (or equivalent). Pasteurisation of raw milk is sufficient to reduce populations of vegetative bacterial pathogens to a level that is considered safe for public health (Cogan, 2003). The study "Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products" concluded that there is ample heat resistance data to indicate that the vegetative cells of the most significant milk-borne pathogens are destroyed by pasteurisation, with a reasonable margin of safety (FSANZ, 2007).

(Cogan, 2003). This can lead to undesirable consequences, such as the production of *Staphylococcus* enterotoxins that will remain in the cheese.

The rate of pH reduction (time to reach desired pH) is fairly characteristic of the cheese variety being manufactured and can range from 5 - 6 hours for Cheddar and Cottage type cheeses to 10 - 12 hours for Dutch and Swiss types (Fox *et al.,* 2000). The production of acid at the appropriate rate and extent is critical to the microbiological safety of cheese and is dependent upon the amount of starter used and the temperature profile of the curd. Reaching the appropriate end point pH in acidification plays a critical role in reducing the growth and level of microbial pathogens in the final cheese. Most bacteria require a neutral pH value for optimum growth and grow poorly at pH values below 5.0. The pH of the curd for most hard cheese varieties is in the range 5.0 -5 .4 but it is approximately 4.6 for the soft, acid-coagulated varieties (Cottage, Quarg and cream cheese) and some rennet-coagulated varieties (*e.g.* Camembert and Brie). The pH limits required for growth of selected pathogens are listed in Table 16. It should be noted that the minimum pH required for growth is influenced by other factors such as temperature, water activity and salt concentration.

Table 16: pH limits required for growth for various pathogens (ICMSF, 1996)

Pathogen	Minimum	Optimum	Maximum
Campylobacter	4.9	6.5 – 7.5	ca. 9
Salmonella spp.	3.8	7 – 7.5	9.5
L. monocytogenes	4.4	7.0	9.5
S. aureus	4.0	6 - 7	10
E. coli	4.4 2.5-3.0 (acid tolerant strains)	7 – 7.5	9.5

9.1.3.2 Coagulation

During coagulation, milk proteins (casein) are clotted, entrapping the milk fat, water and water-soluble components. Coagulation may be achieved by (Fox *et al.,* 2000):

- Limited proteolysis by selected proteinases (rennets)
- Acidification to pH 4.6 (the isoelectric point of casein)
- Acidification to a pH value more than 4.6 (perhaps ~5.2) in combination with heating to roughly 90°C

The vast majority of cheeses are enzymatic (rennet) coagulated. Acid-coagulated varieties include Cottage and Quarg cheeses and the acid heat-coagulated cheeses are usually produced from whey or a blend of whey and skim milk (Ricotta and related varieties).

Coagulation is temperature dependent and cow milk does not coagulate at temperatures less than 18°C (Fox *et al*., 2000). Coagulation is normally carried out at the optimum growth temperature of mesophilic starter cultures, which vary between 32 - 37°C (Broome *et al*., 2003), although the optimum temperature for coagulation is 40 - 45°C. The temperature employed to facilitate coagulation in cheesemaking is often referred to as the curd setting temperature.

The process of warming and holding milk at coagulation temperatures of 32 - 37°C is favourable for the growth of pathogenic microorganisms if they are present in the milk (Table 17). If the starter culture is slow, non-viable, or if phage or antibiotics are present in

the milk and impact on the starter culture, a rapid increase in the population of other microorganisms in the milk, including pathogens is possible.

The minimum, maximum and optimum temperature conditions for the growth of selected pathogens are listed in Table 17.

Table 17: Temperature growth conditions for various pathogens (ICMSF, 1996)

Pathogen	Minimum (°C)	Maximum (°C)	Optimum (°C)
L. monocytogenes	-1.5	45	37
Salmonella spp.	7	46	35-43
E. coli	7	48	37
Campylobacter spp.	30	45	42-43
S. aureus	7	48	35-40
S. aureus (toxin production)	10	48	40-45

9.1.3.3 Curd cutting and cooking operations

The final characteristics (*e.g.* moisture content) of a cheese are largely determined by the extent of syneresis (expulsion of whey) after coagulation. Enzymatic (rennet) or acid-coagulated milk gels are quite stable if left undisturbed, but if cut or broken they rapidly undergo syneresis, expelling whey. Syneresis essentially concentrates the fat and casein of milk by a factor of 6 – 12, depending on the cheese type (Fox *et al*., 2000). An increase in the concentration of pathogens may occur after expulsion of the whey due to entrapment within the curd matrix.

The rate and extent of syneresis are influenced by: milk composition, especially the concentrations of calcium and casein; the pH of the whey; curd cooking temperature; the rate of stirring of the curd-whey mixture and time.

Cooking of the curd is a cheesemaking step that slows and stops the growth of the starter culture, facilitates the contraction of the curd and subsequent expulsion of whey. Curd cooking temperatures vary according to the type of cheese and the way acidification is carried out. For soft and semi-soft cheese the curd cooking temperature ranges from 30 - 38ºC (Banks, 2003; Van den Berg., 2003); for hard cheese the curd cooking temperature ranges from 38 - 55ºC (Bachmann *et al*., 2003); and for acid/heat coagulated cheeses such as Cottage cheese, Cream cheese, Quark, Queso Blanco, Ricotta, Mascarpone and Paneer cheese, curd cooking temperatures can be as high as 90°C (Fox, 2004; Lucey, 2003).

Although there are certain common features, the factors that promote and regulate syneresis in a cheese variety or family of varieties are specific to that variety or family. In the case of Cheddar and Swiss-type cheeses, dehydration is accomplished mainly in the cheese vat by fine cutting the coagulum, extensive "cooking" of the curds-whey mixture (to ~40°C for Cheddar and ~55°C for Swiss-type cheeses) and vigorous agitation during cooking (Fox *et a*l., 2000). For the softer (high moisture) varieties, the milk gel may be scooped directly into the moulds without cutting or cooking, with whey explosion occurs mainly in the moulds as the pH decreases. Curds for some varieties (*e.g.* Cheddar and Swiss) are subjected to considerable pressure in the moulds to aid whey removal, while curds for the softer varieties are pressed only under their own weight.

Where the curd cooking temperature is below 48°C microbial pathogens may grow (Table 18), until the acidity of the curd becomes sufficiently high. Once temperatures are above 48°C there are lethal effects on microbiological pathogens such as *Campylobacter* spp., *E. coli, L. monocytogenes, Salmonella* spp. and *S. aureus*. Table 18 lists the effect of temperatures above 50°C (D-values) on these pathogens.

Table 18: Effect of temperatures greater than 50°C on pathogens (ICMSF, 1996)

Organism	Effect of temperature
C. jejuni	D_{50} = 1.3 – 5.4 minutes (skim milk) D_{55} = 0.7 - 1 minutes (skim milk)
Pathogenic *E. coli*	D_{50} = 31 minutes (buffalo milk) D_{55} = 5.5 minutes (skim milk) D_{55} = 6.6 minutes (whole milk) $D_{57.2}$ = 1.3 minutes (raw milk)
L. monocytogenes	D_{50} = 31.67 minutes (reconstituted non-fat dry milk) $D_{52.2}$ = 24 minutes (sterile, whole milk) $D_{52.2}$ = 37 minutes (commercially sterile, whole milk) D_{55} = 4.5 minutes (reconstituted non-fat dry milk) $D_{57.8}$ = 6.25 minutes (raw skim milk) $D_{57.8}$ = 4.83 minutes (raw whole milk)
Salmonella Typhimurium	$D_{51.4}$ = 49 minutes (laboratory media + 10% milk solids) $D_{54.7}$ = 7.5 minutes (laboratory media + 10% milk solids) $D_{55.2}$ = 4.7 minutes (laboratory media + 10% milk solids) $D_{55.7}$ = 3.2 minutes (laboratory media + 10% milk solids)
S. aureus	D_{50} = 10 minutes (milk) $D_{54.5}$ = 27 minutes (10% reconstituted skim milk) D_{55} = 3 minutes (milk)

9.1.3.4 Salting

Salting is the last manufacturing operation in cheesemaking. The presence of salt in the curd arrests starter culture growth and promotes syneresis. The level and time of salting also has a major influence on pH changes within the cheese.

Some varieties of cheese, such as Cheddar, are salted by mixing dry salt with the cut curd toward the end of manufacture. The pH of curd for these varieties must be close to the ultimate value (~pH 5.10) at salting (Fox *et al.,* 2000).

However, most cheese varieties are salted by immersion in brine or by surface application of dry salt. Salt diffusion is a relatively slow process and thus there is ample time for the pH to decrease to about 5.0 before the salt concentration becomes inhibitory throughout the interior of the cheese. The pH of the curd for most cheese varieties (*e.g.* Swiss-type, Dutch, Tilsit and blue cheese) is 6.2 - 6.5 at moulding and pressing, but decreases to around 5 - 5.2 during or shortly after pressing and before salting (Fox *et al.,* 2000).

In brine-salted cheeses, the salt concentration is influenced directly by the size of the cheese, brine concentration, brine temperature, and the length of time the cheese is immersed in the brine.

The level of salt (% w/w) in different types of cheese ranges from approximately 0.7 - 7% (Fox *et al*., 2000). Salt, along with pH, redox potential and water activity contribute to the minimisation of spoilage and prevention of pathogen growth in cheese (Fox *et al.,* 2000). However, during the initial phase of cheesemaking, salt is not distributed evenly throughout the curd mass. Dry salt applied to the surface of the milled curd requires time to diffuse throughout the curd mass, hence microorganisms in the curd may initially continue to grow.

With the exception of *S. aureus* and *L. monocytogenes,* most pathogenic microorganisms will not grow when the salt concentration reaches 4% (% w/w). *S. aureus* can grow in the presence of 6.5% of sodium chloride and *L. monocytogenes* can grow in the presence of 10% sodium chloride (Cogan, 2003).

In most cheese varieties, salt concentrations attain levels of 1.6 - 2.5% (%w/w) which is insufficient to inhibit the growth of most microbiological pathogens in cheese (Sphar and Url, 1994).

9.1.4 Effect of ripening (maturation) on cheesemaking

Ripening, maturation or curing are all terms referring to a complex set of biochemical reactions which modify the flavour, aroma and texture of the curd during storage under specific time and temperature conditions post-manufacture. The unique characteristics of individual cheeses developed as a result of ripening are largely influenced by the manufacturing process, that is, by the composition (especially moisture, NaCl levels, and pH), the level of residual coagulant activity, the type of starter, and in many cases by secondary inocula added to or gaining access to the milk or curd.

Although rennet-coagulated cheese varieties may be consumed as fresh cheese at the end of manufacture, most rennet-coagulated cheeses are ripened for a period ranging from about 3 weeks (*e.g.* Mozzarella) to more than 2 years (*e.g.* Parmigiano Reggiano or extra mature Cheddar). Ripening usually involves a change to the microflora of the cheese, including death and lysis of the starter cells, and development of adventitious non-starter microflora (*e.g. Propionibacterium freudenreichii* subsp. *shermanii* in Swiss-type cheese, moulds in mould-ripened varieties and a complex Gram-positive bacterial microflora on the surface of smear-ripened cheeses) (Fox *et al.,* 2000). Generally, the duration of ripening is directly related to the moisture content of the cheese and inversely related to its salt content.

The water activity is lowered during cheese ripening as the cheese loses moisture and the added salt binds free moisture and makes it unavailable for bacterial growth. The hydrolysis of proteins to peptides and amino acids, and of lipids to glycerol and fatty acids during ripening further reduces the availability of water. In addition, organic acids (lactate, acetate, and propionate) are dissolved in the moisture of the cheese and reduce the vapour pressure.

Evaporation of water from the cheese surface during ripening also contributes to the reduction of the water activity of cheese. Gruyere has a faster rate of decrease in water activity during ripening compared to Emmentaler, most likely due to the surface salting of Gruyere during ripening. In addition, the water activity of cheese can vary throughout the cheese itself. Variations are much greater in large cheeses, like Emmentaler than in small cheeses like Appenzeller. This is due to several factors, including the temperature gradient in the cheese during the early stages of fermentation, the loss of moisture during ripening, the NaCl

gradient in the cheese and microbial activity on the rind (Fox *et al.,* 2000). Typical water activity values for various cheeses are listed in Table 19.

Table 19: Typical water activity values for various cheeses (Fox *et al.,* 2000)

Cheese variety	Typical water activity
Parmesan	0.917
Sbrinz	0.940
Gruyere	0.948
Gouda	0.950
Appenzeller	0.960
Tilsiter	0.962
Gorgonzola	0.970
Emmentaler	0.972
Brie	0.980
Camembert	0.982
Cottage cheese	0.988

Pathogenic bacteria are susceptible to reduced water activity. As the water activity falls there is a lengthening of the lag phase, longer generation time and a reduction in the maximum number of cells produced. Cheeses with relatively high water activity (*e.g.* soft cheeses) may readily support the growth of pathogenic bacteria compared to a low moisture cheese. Once the water activity falls to below 0.92, the growth of bacterial pathogens will be inhibited, with the exception of *S. aureus* (Table 20).

Table 20: Minimum water activity for the growth of various pathogens (ICMSF, 1996)

Pathogen	Minimum water activity required for growth
E. coli	0.95
Salmonella spp.	0.94
Listeria monocytogenes	0.92
S. aureus	0.86
Campylobacter spp.	0.99

The temperature at which cheese is ripened is dictated by two opposing requirements: (1) the need to control the growth of potential spoilage and pathogenic bacteria, and (2) the need to promote the ripening reactions and the growth of the secondary microflora (in the case of soft and Swiss-type cheeses).

Higher temperatures promote faster ripening by the starter and non-starter microorganisms but also allow the growth of spoilage and pathogenic bacteria. Generally, Cheddar cheese is ripened at 6 - 8°C, while Camembert and other mould and bacterial smear-ripped cheeses are ripened at 10 - 15°C (Fox *et al.,* 2000). Emmentaler cheese is initially ripened for 2 - 3 weeks at a low temperature (~12°C) after which the temperature is increased to 20 - 24°C for 2 - 4 weeks to promote the growth of propionic acid bacteria and the fermentation of lactate to propionate, acetate and CO_2. The temperature is then reduced again to around 4°C. For soft cheeses, the humidity of the environment is also controlled to prevent excessive evaporation of moisture from the cheese surface. Generally, ripening temperatures are sub-optimal for the pathogens of concern, however they are not inhibitory.

The combined effects of pH, salt, moisture concentration and storage temperature act as hurdles to pathogen growth and survival during the ripening phase. Long storage or ripening of cheese under controlled temperatures contributes to the reduction in microbiological populations present, however challenge studies on various cheeses has shown that this is variable for different pathogens (Section 9.3).

Significant growth of pathogenic microorganisms may occur during ripening of soft cheeses because of their relatively high moisture (water activity varies from 0.97-0.99), high pH and the often high ripening temperature (Cogan, 2003). Hard cheeses, by virtue of their low moisture content and long maturation periods, are unlikely to support the survival and proliferation of microbial pathogens; however this has been shown to be variable.

9.1.5 Effects of yeasts and moulds in cheese

The growth of yeasts and moulds in raw milk cheese can influence the growth and survival of pathogens by their effect on the physicochemical environment of the cheese. These effects may differ between surface and internal mould ripened cheeses.

9.1.5.1 Surface mould-ripened

For surface mould-ripened soft cheeses (*e.g.* Camembert) spores of *P. camemberti* are either sprayed onto the surface after salting, or added directly to the milk.

In these raw milk cheeses the native flora and physicochemical treatments determine the succession of microflora activity responsible for different functions of the cheese ripening process (*e.g.* texture, flavour, colour etc). Starter cultures, by reducing the pH, will select acidophilic microorganisms such as yeasts and filamentous fungi to grow. The mould *Geotrichum candidum* appears at the same time but its growth is limited by salting.
The surface fungal flora (*i.e.* yeast, *Geotrichum* and *Penicillium*) use lactic acid for their growth. Consequently, there is a marked increase in the external pH and an internal migration of lactate towards the surface of the cheese. The surface pH increases steadily to about 7.0 at the end of maturation while the increase is slower in the interior with final pH about 6.0 (Spinnler and Gripon, 2004).

Salting has a selective effect on mould and influences sporulation, germination and growth. Too much salting limits growth of *G. candidum* while the growth of *P. camemberti* is much less affected. Too little salt, combined with insufficient draining causes excessive growth of *G. candidum* and hinders the implantation of *Penicillium* spp. Salting also influences the activity of *Penicillium* enzymes and at 4%, it reduces the degree of proteolysis in camembert.

The changing environmental conditions of pH, salt concentration and water activity induced by the growth of yeasts and moulds in surface mould-ripened cheeses impacts on the growth of pathogens.

9.1.5.2 Internal mould-ripened

Internal mould-ripened cheeses (*i.e.* blue cheese) are characterised by growth of *P. roqueforti.* The microenvironment is generally heterogeneous with pronounced gradients of pH, salt, water activity etc. These parameters and their changes during the course of ripening have a great impact on the growth and biochemical activity of the various microorganisms present in the cheese, including pathogens.

In these cheeses the residual lactose decreases rapidly causing the pH of the interior to rise more rapidly than the surface, as the level of NaCl is lower, and therefore allows faster and earlier growth of the mould cultures. The increase in pH is due to the metabolism of lactic acid to CO_2 by yeasts and moulds and the increased proteolysis, leading to production of NH_3 from amino acids.

Salting (immersion or dry salting) creates a NaCl gradient from the surface to the core which equilibrates slowly during ripening. Overall salt content ranges from 2 - 5 %. The diffusion of salt into core is faster in the piercing channels and in areas with fissures, creating an even more imbalanced salt distribution.

During ripening, the concentration of NaCl and the extent of lipolysis and proteolysis, especially the increase in low molecular weight peptides, significantly influence the water activity. Fat content impacts on cheese structure and hence the diffusion coefficient of NaCl, and also influences the equilibration of water activity throughout the cheese. These conditions will impact on the growth of pathogens in the cheese.

9.1.6 Other factors affecting growth of pathogens in cheese

The native microflora, natural inhibitory systems in raw milk, oxidation-reduction potential and the production of organic acids and use of additives such as nitrates can also have an inhibitory effect on the growth or survival of pathogens in cheese.

Raw milk contains several inherent antimicrobial systems designed primarily to protect the udder from infection, or confer disease resistance to suckling young (Bramley and McKinnon, 1990). The major microbial inhibitors in raw milk are lactoferrin and the lactoperoxidase/thiocyanate/hydrogen peroxide system (LP system). Lesser ones include; lysozyme, specific immunoglobulins and folate and vitamin B12 binding systems.

Lactoperoxidase is a naturally occurring enzyme in milk, and in the presence of hydrogen peroxide and thiocyanate it has a bacteriostatic effect on many organisms, and a bactericidal effect against some specific Gram-negative bacteria *i.e. Pseudomonas* spp. and *E. coli*. Lactic acid bacteria, coliforms and various pathogens are inhibited to some extent by this system (Doyle et al, 1997).

Natural levels of hydrogen peroxide and thiocyanate in raw milk are insufficient to activate the LP system and require addition. Once activated, its effect has limited duration which is influenced by the initial bacterial load, the species and strains of contaminating bacteria, and the storage temperature of the milk.

An expert consultation on the LP system found it has a role to play as part of an integrated system to improve milk quality and safety. However, the system is not considered a replacement for existing technologies, such as cooling and heat treatment. Rather it provides complimentary alternatives to refrigeration, particularly at the primary production stage, when such approaches are not available, feasible or suitable, such as in developing countries (FAO/WHO, 2006).

The effect of competitive organisms has been demonstrated in several studies. Wang *et al*, (1997) inoculated a 5-strain mix of *E. coli* O157:H7 into raw and pasteurised milk and observed survival and/or growth at 5^{o}C, 8^{o}C, 15^{o}C and 22^{o}C for up to 28 days. During storage at 8^{o}C, growth increased by 2 - 3 log cfu/ml in raw milk compared to approximately 4 log cfu/ml in pasteurised milk after day 7. Growth rate increased with increasing temperature in both the raw milk and pasteurised milk. Overall, the study demonstrated that although growth is slower in raw milk than in pasteurised milk, *E. coli* O157:H7 can grow at 8^{o}C with populations increasing by 1- 2 log cfu/ml within 4 days.

L. monocytogenes was shown to have a longer lag phase and to grow more slowly (1.9 times) in Camembert cheese made from raw milk compared to cheese made with pasteurised milk (Meyer-Broseta *et al*., 2003).

The efficacy of organic acids as inhibitors of microbial growth is thought to depend on the amount of undissociated acid present and therefore on the dissociation constant and pH. The dissociation constant of propionic, acetic and lactic acids (the principal acids found in cheese) are 4.87, 4.75 and 3.08, respectively. At the same concentration lactic acid is the least and propionic acid the most effective inhibitor (Fox *et al.,* 2000). Propionic acid is very effective at repressing the growth of moulds. However, the concentration of the acid is also important, and lactate is invariably present at much greater concentrations in cheese and cheese curd than either of the other two acids. The pH of mould- and smear-ripened cheeses characteristically increases during ripening, particularly on the surface, due to the growth of yeast and moulds.

Nitrate is added to the milk for some cheeses, especially Dutch-type cheeses like Gouda and Edam to prevent the production of early and late gas by coliforms and *Clostridium tyrobutyricum*, respectively. However much of the nitrate is lost in the whey.

The oxidation-reduction potential is a measure of the ability of chemical or biochemical systems to oxidize (lose electrons) or reduce (gain electrons). The exact mechanism by which the oxidation-reduction potential of cheese is reduced is not clear. However, it is almost certainly related to the fermentation of lactose to lactic acid by the starter culture during growth and is probably related to the reduction of the small amounts of oxygen in the milk to water. Because of these reactions, cheese is essentially an anaerobic system in which only facultatively or obligately anaerobic microorganisms can grow. The bacteria that develop on the surface of cheese are mainly obligate aerobes and are unable to grow within the anaerobic cheese environment.

9.1.7 Environmental contamination

Raw milk and raw milk cheeses may be subject to contamination from the environment, equipment, personnel or raw ingredients. Pasteurised milk cheeses are also exposed to similar contamination. However, there is a higher risk of environmental contamination for raw milk cheese products, as there is greater opportunity for pathogenic microorganisms to contaminate and colonise the environment because of the introduction of raw milk into the processing plant.

9.1.7.1 Environment and equipment

Contamination from microbiological hazards may arise from the production environment at any of the cheesemaking steps including coagulation, curd cooking, cutting, milling, hooping, salting, ripening and packaging.

Equipment (*e.g.* baskets/forms, mats, wooden ripening benches, etc) used during the manufacture of cheese contribute to the diversity of the microflora of the cheese, but may also be a vehicle for pathogen contamination. Contamination of cheese can result from inadequately cleaned and sanitised equipment. The soil encountered in cheesemaking plants consists mainly of milkfat, protein and milk minerals. Inadequate cleaning allows microorganisms to adhere and grow on equipment surfaces and form protective extracellular matrices – biofilms. Once formed, elimination of biofilm is very difficult. Dead ends, corners, cracks, crevices, gaskets, valves and joints in cheesemaking equipment are vulnerable to biofilm accumulation and consequently transfer of microbiological hazards to cheese. Continuous cheesemaking operations, if not cleaned adequately between batches, are particularly vulnerable to this type of contamination.

Of particular importance is *L. monocytogenes*, which is a significant environmental microbial contaminant in cheesemaking plants. Its psychrotrophic nature enables the organism to colonise and grow in wet and cold environments. This includes condensation on walls and ceilings, equipment surfaces, drains, floor puddles, condensate collected in refrigeration units and condensation in compressed air lines (Nelson, 1990).

9.1.7.2 Personnel

The poor hygiene and health status of cheesemaking personnel can lead to contamination of cheese during manufacture. The microflora on the hands and outer garments of food handlers generally reflects the environment and habits of the individuals. This flora would normally consist of organisms found on any object handled by the individual as well as those picked up from dust, water, soil etc. Some pathogens are specifically associated with the hands, nasal cavities and mouth of personnel. For example, *Micrococcus* spp. and *Staphylococcus* spp. from skin (particularly cuts and wounds) and upper respiratory tissues may contaminate raw milk cheese during manufacture (ICMSF, 1998).

Other pathogens that may be transferred to cheeses include intestinal pathogens such as *Salmonella* spp. and *Shigella* spp. which can be deposited onto equipment and surfaces via soiled hands (Adams and Moss, 1995).

Both raw milk and pasteurised milk cheeses may be contaminated with microbiological hazards by personnel.

9.1.7.3 Raw materials

Ingredients, other than raw milk, used in the manufacture of cheese include starter cultures, rennet, salt and other additives. Additionally, a range of other raw materials can be added to cheeses during their manufacture including vine leaves, herbs and spices, which may introduce contamination.

Where starter cultures are propagated in the cheesemaking factory, there is a greater risk of bacteriophage which may result in slow fermentation and acidification. Typically smaller and artisanal cheesemakers may practice "backslopping" [28],or propagate their own starter culture lines.

Starter cultures may be added in liquid, frozen, lyophilized or dried form. Starter cultures produced by commercial suppliers are produced under controlled conditions and contamination risks are minimised. Starter cultures which are reproduced daily at the cheese plant by some form of backslopping present a higher risk of contamination. No special precautions are used to prevent contamination from raw milk or the environment when producing or using natural starter cultures with limited control during starter reproduction (Fox *et al*., 2004).

During commercial preparation, rennets undergo a series of extraction and purification steps and NaCl is added to rennets to inhibit microbial growth during storage (Beresford and Williams, 2004). While salt is unlikely to introduce pathogens into the cheese curd, rubbing dry salt on to the cheese surface results in the transfer of microorganisms from the cheese maker's hands and the environment to the cheese surface (Beresford and Williams, 2004).

Industrial brines are used repeatedly and are infrequently pasteurised. The salt content of most brines is maintained at 18 – 24% for most cheese varieties although for Feta it is 5 - 10% (Larson *et al*., 1999). Brine temperatures are generally maintained at 4 - 10°C (Larson *et al.,* 1999). While the relatively high salt content of brine inhibits the growth of most microorganisms, leaching of proteins and other nitrogenous compounds from the cheese into the brine may enhance the survival of microorganisms that gain access to the brine. Brining tanks used in cheesemaking may be vectors for contamination by *L. monocytogenes*. Studies have also shown that *S.* Typhimurium and *E. coli* O157:H7 can survive for several weeks under typical brining conditions (Ingham *et al*., 2000).

9.1.8 Summary of effects on microorganisms during cheesemaking

Cheesemaking consists of a number of different processing steps and factors which have an effect on the growth and survival of microorganisms. While each of these hurdles has an effect, it is the collective effect that has the greatest impact on the growth, survival or inactivation of microbial pathogens in cheese.

Survival of pathogenic microorganisms is also dependent on a number of factors including: the initial population in raw milk; their physiological condition; strain characteristics such as tolerance of low pH, salt, reduced water activity and heat, and resistance to bacteriocins produced by lactic acid bacteria, etc.

[28] "Backslopping" is the use of an old/existing batch of fermented product to inoculate a new one.

Cheesemaking steps following harvesting of milk, which act as hurdles against the growth and/or survival of pathogenic microorganisms include: pre-heat treatments of raw milk, acidification, curd cooking and the time/temperature of ripening. Factors influencing the growth of microorganisms in cheese include water activity, salt concentration, oxidation-reduction potential, pH and nitrate.

The greatest lethal effect on pathogens is achieved through the application of heat[29], either to the raw milk or the cheese curd (*e.g.* pasteurisation, thermisation and curd cooking). Milk used in the manufacture of raw milk cheese does not undergo a pre-treatment heat process such as pasteurisation or thermisation, but may undergo curd cooking. However, minor heating during coagulation (32 - 37°C) is favourable for the growth of pathogens if present. If the starter culture is slow, non-viable, or if phage or antibiotics are present in the milk, a rapid increase in the population of pathogens is possible. As such, the microbiological quality of the raw milk is paramount in ensuring the safety of all raw milk cheeses.

Reaching the appropriate end point pH during acidification also plays a critical role in minimising the growth of pathogens during cheesemaking. The ability of the starter culture to dominate (active growth of the lactic acid starter bacteria) in milk reduces the likelihood of growth of other microorganisms. In particular, rapid acid production inhibits growth of *S. aureus* and therefore prevents development of staphylococcal enterotoxin.

During ripening/maturation the combined effects of pH, salt, reduced moisture and storage temperature lead to a reduction in water activity and thereby further inhibit pathogen growth and promote pathogen die-off. Other compounds produced during curd manufacture and ripening (*e.g.* H_2O_2 and fatty acids) also inhibit microbial growth, but the concentration of these compounds, produced by starter cultures, are insufficient to have a significant effect on microbial growth.

While cheesemaking involves several hurdles that influence the growth and/or survival of pathogenic microorganisms in cheese, their efficacy varies between cheese types and varieties. The extent of pathogen die-off achieved during the ripening period is also extremely variable and depends upon the specific physicochemical characteristics of the cheese and the properties of the microorganism. Hence, it is a combination of hurdles, rather than an individual processing step or physicochemical property that has the greatest impact on pathogen survival. Pathogens will grow more easily in cheese with a high moisture content, neutral pH and low salt content, compared to the more hostile environment of the high temperature curd cooked cheeses which are ripened over a prolonged period *e.g.* extra-hard cheese.

Further contamination of raw milk cheese may arise from the environment, equipment, personnel or other raw materials. However, the risk of contamination from these sources is no different to that encountered by cheeses made from pasteurised milk.

[29] Temperatures above 48°C are generally lethal to pathogens such as *L. monocytogenes, Salmonella* spp., *E. coli, Campylobacter* spp. and S. *aureus* (ICMSF, 1996).

9.2 Contamination of raw milk cheese post-manufacture

Cross-contamination of raw milk cheeses with microbiological hazards can occur through inadequate food handling practices during retail sale, food service and in the home. Unpackaged cheeses in delicatessens are particularly vulnerable to cross contamination, especially with *L. monocytogenes* from other foods, food utensils, from display cabinet surfaces and condensation.

Contamination of cheese post manufacture during retail, food service operations, or in the home can result from poor hygiene or infected food handlers. Pathogens and viruses can be transmitted to food via the faecal-oral route from hands soiled with faeces (Adams and Moss, 1995).

Storage time and temperature during retail display, food service and in the consumer household may impact on the number of microorganisms present in raw milk cheeses. In those cheeses that support the growth of *L. monocytogenes*, storage times and temperatures may have an important impact on cheese safety.

Improper storage of dairy products may allow growth of pathogenic microorganisms to levels likely to cause illness. Spores, which have survived processing, may germinate if storage temperature and times are not controlled. Furthermore, low levels of pathogens which may have been introduced through environmental contamination during processing, may also grow if storage temperatures and time are not controlled (*e.g. Salmonella* spp.). Correct storage (refrigeration) of dairy products throughout the transportation and retail supply chain and through to the consumer is important to maintain safety, shelf-life, and quality.

Published and unpublished data obtained from surveys in Australia and overseas consistently show the refrigerated retail cabinet as a weak link in the cold chain. Data on retail storage temperatures during a Meat and Livestock Australia study showed that while the majority of temperatures recorded were below 5°C, some temperatures were as high as 15°C (Meat and Livestock Australia, 2006).

There is a lack of available data on storage conditions in the food service sector, however, temperature control of food stored in domestic refrigerators in Australia is generally poor. In a 1998 survey, 36% of Australian domestic refrigerators (171 samples) had their fresh-food compartments above 5°C for greater than 50% of the time (Jay *et al*., 1998). Data on temperatures in domestic refrigerators during the Meat and Livestock Australia study (Figure 8) confirm this finding (Meat and Livestock Australia, unpublished data).

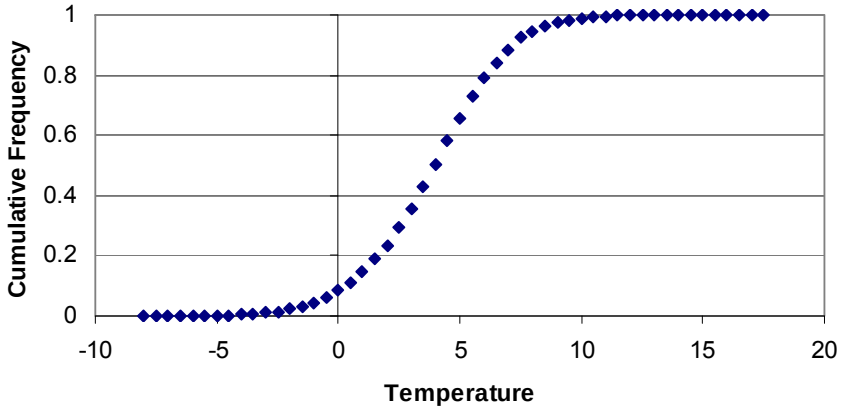


Figure 8: Cumulative frequency distribution for domestic refrigerator temperatures in Australia

9.3 Challenge studies on effects of ripening/maturation on pathogenic microorganisms

Microbiological challenge studies are an important tool for determining the ability of a cheese to support the survival and/or growth of pathogens. There are a number of studies that have been undertaken to examine the behaviour of pathogens such as pathogenic *E. coli*, *L. monocytogenes* and *Salmonella* spp. in various cheese types and the results are summarised in Tables 21, 22 and 23, respectively.

The findings of the challenge studies highlight the variability in survival and/or growth of pathogens in different cheese types, and also demonstrate the variability in processing and maturation conditions of cheeses of the same type.

Most challenge studies which show the survival of pathogens have been based on the use of pasteurised or ultra high temperature (UHT) milk rather than raw milk. The fastest growth rate of specific pathogens has been observed in UHT milk, followed by pasteurised, heat-treated and raw milks (Northolt *et al*., 1988; Rajkowski *et al*., 1994). Therefore, challenge studies which assess the survival of pathogens inoculated into pasteurised milk, may overestimate survival during ripening and maturation due to a lack of competitive microflora. For example, *L. monocytogenes* was shown to grow more slowly (1.9 times) in Camembert cheese made from raw milk compared to cheese made with pasteurised milk. The lag phase for *L. monocytogenes* was also shown to be longer (2.3 times) in Camembert cheese made from raw milk compared to that made with pasteurised milk (Meyer-Broseta *et al*., 2003).

Pathogenic *E. coli* has been shown to survive in Cheddar, Colby, Brick, Feta and Camembert cheeses, despite a reduction in numbers during ripening and maturation (Ramsaran, 1998). However, other studies have shown complete inactivation of *E. coli* in various cheeses including Romano and Tilsiter, and in some cases the same type of cheese *e.g.* Feta (Govaris, 2002).

L. monocytogenes has also been shown to survive in Cheddar, Feta and Camembert cheeses, despite a reduction in numbers during ripening and maturation (Ramsaran, 1998).

Challenge studies have reported variable results for the reduction in *Salmonella* spp. during ripening and maturation of various cheeses (Table 23).

This variability in results is typical in microbiological challenge studies. Caution must be exercised when interpreting data on the survival of pathogens inoculated into milk used for making cheese. Interpretation of challenge study data must take into account a number of factors including: the selection of appropriate pathogens or surrogates; the level of challenge inoculum; the inoculum preparation and method of inoculation; the stage of growth of the inoculum; the duration of the study; formulation factors and storage conditions; and sample analyses.

Table 21: Decrease in *E. coli* numbers during ripening and maturation of cheese

Cheese	*E. coli* strain	% Salt in water	pH	Log decrease	Ripening conditions	Reference
Colby	ETEC-a	3.7 - 4.9	4.9 - 5.3	>3	6.5 wk/10°C	(Kornacki and Marth, 1982)
Colby	ETEC-b	3.9 - 4.0	5 - 5.6	1.5 - 4	11 wk/10°C	(Kornacki and Marth, 1982)
Colby	EIEC-a	5.4 - 5.9	5.3 - 5.5	>5	3.5 wk/10°C	(Kornacki and Marth, 1982)
Colby	O157 EHEC		4.6	4	4 wk/13°C	(Hudson *et al*., 1997)
Cheddar	3 strains O157	3.15	5 - 5.2	2.8 - 5.8	22.5 wk/6 - 7° C	(Reitsma and Henning, 1996)
Cheddar	3 strains O157	3.34	5 - 5.2	ca 2.1	18.5 wk/ 6 - 7°C	(Reitsma and Henning, 1996)
Cheddar	K12 (ATCC 35695)	-	-	<1 3 - 4	60 d/7°C 180 d/7°C	(Teo *et al*., 2000)
Cheddar	O157:H7	-	-	1 1-2	60 d/7°C 180 d/7°C	(Teo *et al*., 2000)
Cheddar	5 strain 0157:H7	3.34 - 4.66	5.28	1-2	26 wk/6 - 7°C	(Schlesser *et al*., 2006)
Brick	ETEC-b	-	5.1 - 5.3	0.64 – 2.4	2 wk, 15.5°C + 5 wk,7°C	(Frank *et al*., 1978)
Tilsiter	NCTC 9001	3.13	5.2 - 5.4	6.5	30 d/11 - 13°C	(Bachmann and Spahr, 1995)
Romano	O157 EHEC	-	5.2 - 5.7	>4.5	2 d,10°C + 30 d,13°C	(Hudson *et al*., 1997)
Camembert	O157:H7	-	5.9	1.2	2 d/8°C + 10 d/12°C + 65 d/2°C	(Ramsaran, 1998)
Feta & Telemes	O157:H7	-	4.6	>5	36 - 44 d/4°C	(Govaris, 2002)
Feta	O157:H7	-	5.3	1.33	75 d/2°C	(Ramsaran, 1998)

Time is from the start of ageing-maturation except for Colby cheese (Hudson *et al*., 1997) where time and extent of decrease is from salting; Romano - after 65 hours in 22% brine; Brick - after 24 hours in 22% brine; Tilsiter - after 24 hours in 20% brine and 1 day ripening at 11 - 13°C

Table 22: Decrease in *L. monocytogenes* numbers during ripening and maturation of cheese

Cheese	*L. monocytogenes* strain	% Salt in water	pH	Log decrease	Ripening conditions	Reference
Parmesan	California (serotype 4b) V7 (serotype 1)	-	6.1 – 6.2	4 - 5	1 - 16 wk	(Yousef and Marth, 1990)
Italian Grana	-	-	5	-	-	(Pellegrino and Resmini, 2001)
Swiss Tilsit	FAM 871584 (serotype 4b)	3.13	5.2 - 5.4	<1	30 d/11-13°C	(Bachmann and Spahr, 1995)
Cheddar	Scott A, V78:CA	-	-	2	75 - 105 d/13°C	(Ryser and Marth, 1987b)
Brick (surface ripened)	Scott A; Ohio; V8; California	-	6.8 - 7.4	1 - 7	20 wk/10°C	(Ryser and Marth, 1989b)
Camembert	-	-	-	4 increase	45 d/6°C	(Ryser and Marth, 1987a)
Blue	Scott A; Strain CA	11.52	5.7 - 6.2	2.6 - 2.7	84 d/9 - 12°C	(Papageorgiou and Marth, 1989b)
Camembert	- (bioluminescent)	-	5.9	0.2	2 d/8°C + 10 d/12°C + 65 d/2°C	(Ramsaran, 1998)
Feta	-	-	5.3	0.8 increase	75 d/2°C	(Ramsaran, 1998)

Table 23: Decrease in *Salmonellae* during ripening and maturation of cheese

Cheese	% Salt	pH	Log decrease	Ripening conditions	Reference
Cheddar	-	5.4- 5.65	2.5	26 wk at 4.5°C	(Hargrove *et al*., 1969)
	-	5.2 - 5.3	5.3	26 wk at 4.5°C	
	-	5 - 5.05	5	13 wk at 4.5°C	
	-	5.2 - 5.4	4	13 wk at 10°C	
Cheddar	-	5.1 - 6	4	14 - 16 wk at 7.5°C	(Goepfert *et al*., 1968)
	-	5.1 - 6	4	10 - 12 wk at 13°C	
Cheddar	2.1 - 2.3	5.2	4.8 - 5.2	20 wk at 7°C	(Mehta and Tatini, 1994)
Samsoe	-	5.2	4	5 - 6 wk at 16 - 20°C + ca 3 wk at 10 - 12°C	(Goepfert *et al*., 1968)
Montasio	-	5.4 - 5.6	ca 4.5	12 - 13 wk at 12°C	(Stecchini *et al*., 1991)
Manchego	2.5 - 3	4.9 - 5.0	ca 7	8 wk at 10°C	(Medina *et al*., 1982)
Manchego	2.5 - 3	4.9 - 5.0	4.6 - ca 6.5	6 wk at 10°C	(Medina *et al*., 1982)
Tilsiter	1.23	5.2 - 5.4	6.3	4 wk at 11 - 13°C	(Bachmann and Spahr, 1995)

Time and extent of decrease in *Salmonellae* in Cheddar is from 1 day after production; in the semi-hard cheeses Montasio from day 3 (after brine-salting) and Tilsiter from day 3 (after brine-salting and 1 day ripening); and for Manchego, from day 2 (after brine-salting). Cheddar used by Mehta and Tatini (1994) had a water activity of 0.95-0.97. Internal salt content of Manchego after 60 d, and for Tilsiter after 90 d.

10. Assessing the safety of selected raw milk cheeses

The risk assessment process used by FSANZ is consistent with Codex, FAO and WHO protocols and involves four distinct steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation.

Individual risk assessments for raw milk extra hard, Swiss-type, Cheddar, blue, Camembert and Feta style cheeses have been undertaken to assess the risk posed to public health and safety from selected microbiological hazards from the consumption of these cheeses. The risk assessments also consider these cheeses when produced from raw cow, goat or sheep milk. Raw milk cheese made of milk from other species was not examined due to insufficient available data.

The microorganisms selected for consideration are representative of those that have been clearly implicated in foodborne illness and may be present in raw milk.

The risk that these microbiological hazards pose to public health and safety from the consumption of the selected cheeses was characterised using a qualitative framework.

Susceptible populations have been described as individuals who may be more susceptible to infection from specific microbiological hazards due to a lowered or reduced immunity and includes the very young and old, the immunocompromised and pregnant women and their unborn children. This assessment uses the term susceptible populations to include all susceptible individuals.

10.1 Summary of raw milk extra hard cheese risk assessment

The qualitative framework was used to characterise the risk to public health and safety from *C. jejuni*, *E. coli* (EHEC), *L. monocytogenes*, *Salmonella* spp. and *S. aureus* following the consumption of raw milk extra hard cheese made from either cow, goat or sheep milk (Appendix 8).

The extra hard cheeses include the well known Italian parmesan style cheeses, such as Parmigiano Reggiano, Grana Padano, Romano, Asiago and Montasio, as well as the Swiss produced Sbrinz cheese. Extra hard varieties can be manufactured from cows', sheep's or goats'milk or mixtures thereof. These cheeses are typically prepared using relatively high curd-cooking temperatures (>45ºC) and long storage/maturation times (8 - 24 months), resulting in low moisture contents, generally less than 35%.

The specific manufacturing processes assessed for Pecorino Romano, Asiago and Montasio cheeses included thermal treatment of the milk (thermisation and pasteurisation). While the significant effect that thermal treatment would have on the pathogens was noted, the risk assessment analysed the effect of the curd cooking processes and the effects of ripening and storage on bacterial reduction. This allowed for an evaluation of the effect of these production processes on pathogen survival for these cheese types if the raw milk used was not subject to thermisation or pasteurisation. For simplicity, the cheeses will be referred to as Romano, Asiago and Montasio.

Key findings

Raw milk extra hard cheeses have a low likelihood of containing *C. jejuni/coli*, *E. coli* (EHEC), *Salmonella* spp., *S. aureus* and *L. monocytogenes*. While these organisms may be present in the raw milk, and in some situations increase in numbers by 1-2 logs during the initial phase of cheesemaking (warming and holding the raw milk when the starter culture is first added), these organisms will be inactivated by conditions during the curd cooking and during the prolonged ripening of this class of cheeses.

Using the qualitative framework, the risk from selected pathogens in raw milk extra hard cheese is characterised as being between negligible and low (see Table 24).

Table 24: Risk characterisation of raw milk extra hard cheese

Pathogen	Hazard characterisation	Exposure assessment	Risk Characterisation
C. jejuni	Very low Low[#]	Negligible	Negligible
E. coli (EHEC)	High	Negligible	Low
Salmonella spp.	Low Moderate[#]	Negligible	Negligible Very Low[#]
S. aureus	Negligible	Negligible*	Negligible
L. monocytogenes	Negligible Moderate[#]	Negligible	Negligible Very low[#]

[#] Susceptible populations

* Very low in low temperature curd-cook extra hard cheeses

Conclusions

The process of manufacturing extra hard raw milk cheeses results in a substantial or complete reduction in microbiological hazards so they represent a **low** to **negligible** risk to both general and susceptible populations. While the risk from raw milk cheeses is based on a "per serve" basis it is estimated the likely consumption would be very low.

The process of manufacturing raw milk extra hard cheese has been assessed to affect selected pathogens as described in Table 25.

Table 25: Risk associated with raw milk extra hard cheese

Pathogen	*Risk associated with raw milk extra hard cheese*
Campylobacter spp.	*Campylobacter* spp. are unlikely to survive processing and maturation and are a **negligible** risk.
E. coli (EHEC)	**Low** risk as the organism doesn't survive the curd cooking process in the high curd cook cheeses or during cheese maturation.
Salmonella spp.	**Negligible** risk (general population) and **very low** risk (susceptible population) as the organism doesn't survive the curd cooking process in the high curd cook cheeses or during cheese maturation.
S. aureus	Risk from *S. aureus* is considered **negligible**. Conditional on good control over animal health and raw milk handling to prevent growth of the organism to numbers where toxin production is possible.
L. monocytogenes	**Negligible** risk (general population) and **very low** risk (susceptible population) as the organism doesn't survive the curd cooking process in the high curd cook cheeses or during cheese maturation.

There is no difference in the public health and safety risk from *Campylobacter* spp., *E. coli* (EHEC), *Salmonella* spp., *S. aureus* and *L. monocytogenes* in raw milk extra hard cheeses made from either cow, goat or sheep milk.

The microbiological safety of Parmigiano Reggiano, Grana Padano and Sbrinz is enhanced by the high temperature curd cook *i.e.* 55 - 57°C which results in significant destruction of pathogens. Where lower cooking temperatures are used (*i.e.* Romano, Asiago and Montasio) there is less destruction. For all of these cheeses, inactivation of pathogens continues throughout ripening (provided the pH is 5.5 or less) and reductions of greater than 5 logs occur when ripening extends beyond 3 months regardless of the curd cooking temperature. Rapid acidification (*i.e.* pH <5.5 after 3 - 6 hours) of all extra hard cheeses is also critical for producing a safe cheese.

The findings of the raw milk extra hard cheeses assessed may be applied to the entire extra hard cheese category as they generally have similar physicochemical characteristics and manufacturing protocols *e.g.* curd cooking and long ripening times.

10.2 Summary of raw milk Swiss-type cheese risk assessment

The qualitative framework was used to characterise the risk to public health and safety from *C. jejuni*, *E. coli* (EHEC), *L. monocytogenes*, *Salmonella* spp., and *S. aureus* following the consumption of raw milk Swiss-type cheese made from either cow, goat or sheep milk (Appendix 9).

Swiss-type cheeses are classified as either hard or semi-hard. They are characterised by propionic acid fermentation leading to the formation of eyes or mechanical openings resulting from the incomplete fusion of curd pieces and the production of CO_2. Swiss-type cheeses may be made using raw or thermised milk[30] and includes varieties such as Emmentaler, Gruyère, Appenzeller, Tilsiter, Vacherin Fribourgeois and Tête de Moine.

The specific manufacturing processes assessed for Appenzeller, Tilsiter and Vacherin Fribourgeois cheeses included thermal treatment of the milk (thermisation). While the significant effect that thermisation would have on the pathogens evaluated is noted, the risk assessment analysed the effect of the curd cooking processes and the effects of ripening and storage on bacterial reduction. This allowed for an evaluation of the effect of these production processes on pathogen survival for these cheese types if the raw milk used was not subject to thermisation.

Key findings

The cheeses Gruyere, Emmentaler, Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois have a low likelihood of containing *E. coli* (EHEC), *S. aureus, Salmonella* spp. and *Campylobacter* spp. Although these organisms may be present in the raw milk and grow during initial stages of cheese manufacture, the processing conditions and physicochemical properties of the cheeses are not conducive to growth and/or survival of these organisms. The possibility exists that *L. monocytogenes* may survive and/or grow in Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses and therefore pose a high risk to susceptible individuals if consumed.

[30] Raw milk heat treated to a minimum temperature of 62°C for a period of not less than 15 seconds.

Using the qualitative framework, the risk from selected pathogens in raw milk Swiss-type cheeses is characterised as being between negligible and low, with the exception of a high risk to susceptible populations from *L. monocytogenes* in Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois (Table 26).

Table 26: Risk characterisation of raw milk Swiss-type cheese

Hazard	Hazard Characterisation	Exposure Assessment	Risk Characterisation
C. jejuni	Very low Low#	Negligible	Negligible
E. coli (EHEC)	High	Negligible	Low
Salmonella spp.	Low Moderate#	Negligible	Negligible Very Low#
S. aureus	Negligible	Negligible	Negligible
L. monocytogenes	Negligible Moderate#	Negligible/Moderate*	Negligible Very low# Low* High*#

\# Susceptible populations
* Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine cheeses

Conclusions

The process of manufacturing raw milk Swiss-type cheeses results in a substantial reduction in the levels of most microbiological hazards and therefore these cheeses represent a **low** to **negligible** risk to the general population. However, *L. monocytogenes* represents a **high** risk to susceptible populations in Swiss Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses. While the risk from raw milk cheeses is based on a "per serve" basis it is estimated the likely consumption would be extremely low.

The process of manufacturing raw milk Swiss-type cheese has been assessed to affect selected pathogens as summarised in Table 27.

Table 27: Associated risk of raw milk Swiss-type cheese

Pathogen	Risk associated with raw milk Swiss cheese
Campylobacter spp.	*Campylobacter* spp. are unlikely to survive processing and maturation and are a **negligible** risk.
E. coli (EHEC)	**Low** risk as the organism doesn't survive the curd cooking process in the high curd cook cheeses or during cheese maturation.
Salmonella spp.	**Negligible** risk (general population) and **Very Low** risk (susceptible population) as the organism doesn't survive the curd cooking process in the high curd cook cheeses or during cheese maturation.
S. aureus	Risk from *S. aureus* is considered **negligible**. Conditional on good control over animal health and raw milk handling to prevent growth of the organism to numbers where toxin production is possible.
L. monocytogenes	**Low** risk for general population. **High** risk for susceptible populations in Swiss Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses. *L. monocytogenes* does not survive the manufacturing process for Sbrinz, Gruyere and Emmentaler cheeses, however, it may grow in the initial stages of manufacturing and *L. monocytogenes* may survive maturation in Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses.

There is no difference in the public health and safety risk from *C. jejuni, E. coli* (EHEC), *Salmonella* spp., *S. aureus,* and *L. monocytogenes* in raw milk Swiss-type cheeses made from either cow, goat or sheep milk.

There is a degree of uncertainty surrounding the fate of pathogens in Gruyere, Emmentaler, Tête de Moine and Vacherin Fribougeois cheeses. Although challenge studies are available for Emmentaler and Tilsiter cheese, no or limited data is available for the other Swiss-type cheeses assessed. The safety of these cheeses has therefore been assessed on the likely effect cheesemaking may have on pathogens, based on processing (curd cooking and maturation conditions) and intrinsic chemical characteristics (such as pH, water activity and moisture content).

The microbiological safety of raw milk Sbrinz, Gruyere and Emmentaler is dependent upon the high temperature curd cook *i.e.* > 52°C which results in significant destruction of these pathogens. For Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois, lower curd cooking temperatures are used and there is less destruction of pathogens. For these cheeses, inactivation of pathogens is also highly dependent upon maturation/ripening. Rapid acidification of Swiss-type cheeses (*i.e.* pH reduced to 5.5 in less than 5 hours, or 8 hours for Gruyere) is critical in producing a safe cheese.

The findings of the raw milk Swiss-type cheeses assessed may be applied to other Swiss-type cheese types as this group of bacterially ripened cheese with eyes (lactate fermentation) have similar physicochemical characteristics and manufacturing protocols. However, the raw milk cheeses assessed cannot be applied to other hard cheeses based on moisture (*i.e.* 37 - 42% moisture) as the moisture content of the cheeses assessed (34 - 44%) overlap between the extra hard and hard moisture categories and do not represent all types of hard cheeses in respect to physicochemical characteristics and manufacturing protocols.

10.3 Summary of raw milk Cheddar cheese risk assessment

The qualitative framework was used to characterise the risk to public health and safety from *E. coli* (EHEC), *L. monocytogenes*, and *S. aureus* following the consumption of raw milk Cheddar cheese made from either cow, goat or sheep milk (Appendix 10).

The fate of these pathogens during the manufacture of a raw milk Cheddar cheese was determined using a probabilistic model developed by the University of Tasmania and adapted by FSANZ.

Cheddar cheese is classified as a hard cheese. Cheddar cheeses generally undergo a mild curd cooking step followed by milling and dry salting. However, Cheddar cheese may be ripened from 4 months to greater than 2 years, and results in significant differences in the physicochemical properties of individual Cheddar cheeses. The manufacturing parameters and physicochemical properties for the modelled raw milk Cheddar cheese are based on experimental data from challenge studies and a maturation time of 6 months. These are provided in Appendix 10 (Figure 1 and Table 1) and summarised in Table 28 below.

Table 28: Modelled raw milk Cheddar cheese manufacturing parameters and physicochemical properties

Step	Parameters/Properties
Acidification	pH 5.7 in 45 - 90 minutes
Curd cooking	38°C for 30 - 45 minutes
Salting	1.5 - 3% dry salt
Ripening period	6 - 7°C for 26 weeks
Final pH	5.2
Final water activity	0.983 - 0.992
Final salt in moisture	2.8%

Key findings

During the production of raw milk Cheddar cheese it was predicted that there would be an overall increase of 0.98 logs in the concentration of *E. coli* from the initial levels in the raw milk to those present in the final cheese. The overall concentration of *L. monocytogenes* in the final cheese was found to decrease by 2 logs compared with the levels initially in the raw milk. However, this predicted net change in concentration was found to be highly variable due to the observed differences in the rate of inactivation between different strains found in challenge studies.

The probabilistic model predicted high inactivation of *S. aureus* during the ripening of Cheddar cheese (>7 log), however, during the initial stages of cheese production there was an estimated 3 log increase. For example if 100 cfu/ml *S. aureus* were present in the raw milk, levels may reach 10^5 cfu/g in the cheese prior to ripening, and this may allow the organism to produce sufficient enterotoxin to cause illness.

Using the qualitative framework, the risk from selected pathogens in this raw milk Cheddar cheese is characterised in Table 29.

Table 29: Risk characterisation of raw milk Cheddar cheese

Pathogen	Hazard	Exposure	Risk Characterisation
E. coli (EHEC)	High	Moderate	High
S. aureus	Negligible	Low	Very Low
*L. monocytogenes**	Negligible Moderate#	Very Low	Negligible Low#

\# Susceptible populations

* For raw milk cheese manufactured from sheep milk the hazard is moderate resulting in a risk characterisation of Low for susceptible populations

Conclusions

The process of manufacturing the modelled raw milk Cheddar cheese does not result in a substantial or complete reduction in *E. coli* or *L. monocytogenes*. *E. coli* (EHEC) therefore represents a potential **high** risk to general and susceptible populations. *L. monocytogenes* represents a **negligible** risk to the general population and **low** risk for susceptible populations. *S. aureus* represents a **very low** risk to general and susceptible populations. As Cheddar cheese is the most commonly consumed cheese in Australia, it is possible raw milk Cheddar cheese could be consumed by all population groups.

The manufacturing parameters used for this raw milk Cheddar cheese have been assessed to affect selected pathogens as described in Table 30.

Table 30: Risk associated with raw milk Cheddar cheese

Pathogen	Risk associated with raw milk Cheddar cheese
E. coli (EHEC)	**High** risk as the organism may survive the cheesemaking process and cheese maturation.
S. aureus	Risk from staphylococcal enterotoxin is considered **very low**. Conditional on good control over animal health and raw milk handling. The organism doesn't survive ripening/ maturation.
L. monocytogenes	**Negligible** risk (general population) and **low** risk (susceptible population groups) as the organism survives the cheesemaking process*.

* For raw milk cheese manufactured from sheep milk the risk is **very low** for general population and **moderate** for susceptible population groups

There is no difference in the public health and safety risk from *E. coli* (EHEC) and *S. aureus* in raw milk Cheddar cheeses made from either cow, goat or sheep milk. However, *L. monocytogenes* presents a greater risk in raw milk Cheddar cheese when produced from raw sheep milk compared to cow or goat milk. This is a result of *L. monocytogenes* having a greater prevalence in raw sheep milk compared to cow and goat milks.

Due to the relatively low predicted inactivation of *E. coli* and survival of *L. monocytogenes* during the manufacture of raw milk Cheddar cheese, the microbiological quality of the raw milk and prevention of any contamination during manufacture are critical to microbiological safety.

Quantitative modelling indicates that in order to produce raw milk Cheddar cheese that would meet current microbiological limits in the Code, the initial concentration of *E. coli* and *L. monocytogenes* in the raw milk would need to be less than 10^{-2} and 10^{-3} cfu/ml, respectively. To produce Cheddar cheese unlikely to have sufficient staphylococcal toxin to cause illness (*i.e.* $<10^5$ cfu/g), the initial concentration in the milk would need to be less than 100 cfu/ml.

The extent that these findings could be applied across the breadth of Cheddar cheese varieties is uncertain. It could be assumed that the same level of risk would apply to all raw milk Cheddar cheeses whose manufacturing specifications lie within the range of those of the modelled raw milk Cheddar cheese. However, the findings of the modelled raw milk Cheddar cheese assessed cannot be applied to other hard cheeses based on moisture (*i.e.* 37 - 42% moisture) as the modelled cheese does not represent all types of hard cheeses in respect to physicochemical characteristics and manufacturing protocols.

10.4 Summary of raw milk blue cheese risk assessment

The qualitative framework was used to characterise the risk to public health and safety from *L. monocytogenes* following the consumption of the modelled raw milk blue cheese made from either cow, goat or sheep milk (Appendix 11).

The fate of *L. monocytogenes* during the manufacture of a raw milk blue cheese was determined using a probabilistic model developed by the University of Tasmania and adapted by FSANZ. There was insufficient data on pathogen reduction for *E. coli* and *S. aureus* during the ripening phase of blue cheese for probabilistic modelling of these organisms to be undertaken.

Blue cheese is characterised by a network of blue and green veins running continually throughout the cheese as a result of the growth of *P. roqueforti*. Many countries have developed their own types of blue cheese, each with different characterisitics and manufacturing methods. Some well-known examples include: Gorgonzola, Danablue, Stilton and Roquefort. Curds are cooked at low temperatures before transfer to moulds. Blue cheese can either be dry or brine salted, and then ripened in aerobic conditions to favour mould growth. The cheese is punctured (needling) to allow oxygen to enter the interior of the cheese for mould growth. Considerable structural differences exist within these cheeses which influences the level and distribution of O_2 and CO_2. The minimum pH of blue cheeses ranges from approximately 4.6 – 4.7 in Danablue and Stilton to 5.15 - 5.30 in Gorgonzola and Cabrales. When mature, cheeses can have pH up to 6.0 - 6.5. The classification system of Scott (1986) categorises these cheeses as "semi-hard" (44 - 55% moisture), however, some blue cheeses, such as Kopanisti cheese, have moisture contents greater than 55% and would be classified as "soft".

The manufacturing parameters and physicochemical properties for the modelled raw milk blue cheese are based on experimental data and do not necessarily reflect commercial manufacturing practices or a particular variety of blue cheese. These are provided in Appendix 11 (Figure 1 and Table 1) and in Table 31 below.

Table 31: Modelled raw milk blue cheese manufacturing parameters and physicochemical properties

Step	Parameters/Properties
Acidification	pH <5 within 24 hours
Curd cooking	35 - 36°C for 45 minutes
Ripening period	9 - 12°C 84 days
Final pH	>6
Final water activity	0.974
Final salt in moisture	11%

Key findings

During the production of the modelled raw milk blue cheese it was estimated that there would be an overall increase of less than 2 logs in the concentration of *L. monocytogenes* from the initial levels in the raw milk.

Using the qualitative framework, the risk from selected pathogens in this raw milk blue cheese is characterised as in Table 32.

Table 32: Risk characterisation of the modelled raw milk blue cheese

Pathogen	Hazard	Exposure	Risk Characterisation
L. monocytogenes	Negligible Moderate[#]	Moderate	Low High[#]

Susceptible populations

Conclusions

The process of manufacturing this raw milk blue cheese does not result in a substantial or complete reduction in *L. monocytogenes*. *L. monocytogenes* therefore represents a **low** risk to the general population, however due to the high severity for susceptible populations the risk for this population is **high**. While the risk from raw milk cheeses is based on a "per serve" basis it is estimated the likely consumption would be extremely low.

The process of manufacturing this raw milk blue cheese has been assessed to affect *L. monocytogenes* as described in Table 33.

Table 33: Risk associated with raw milk blue cheese

Pathogen	Risk associated with raw milk blue cheese
L. monocytogenes	**Low** risk (general population) and **high** risk (susceptible population groups) as the organism may increase in population in the cheesemaking process.

There is no difference in the public health and safety risk from *L. monocytogenes* in this raw milk blue cheese made from either cow, goat or sheep milk.

As the manufacture of raw milk blue cheese may result in an overall increase in *L. monocytogenes* above levels initially present in the raw milk, the microbiological quality of the raw milk and prevention of any contamination during manufacture are critical to microbiological safety.

Quantitative modelling indicates that in order to produce raw milk blue cheese that would meet current microbiological limits in the Code, the initial concentration of *L. monocytogenes* in the raw milk would need to be less than 10^{-5} cfu/ml.

The extent that the findings for the modelled raw milk blue cheese can be applied across the breadth of blue cheese varieties is uncertain. It could be assumed that the same level of risk would apply to those raw milk blue cheeses whose manufacturing specifications lie within the range of those modelled. The findings of the modelled raw milk blue cheese assessed cannot be applied to other semi-soft cheeses based on moisture (*i.e.* 43 - 55%) as the moisture content of blue cheeses vary considerably. Further, the physicochemical characteristics and manufacturing protocols of the modelled blue cheese do not represent all types of semi-soft cheeses.

10.5 Summary of raw milk Feta cheese risk assessment

The qualitative framework was used to characterise the risk to public health and safety from *E. coli* (EHEC), *L. monocytogenes*, and *S. aureus* following the consumption of raw milk Feta cheese made from either cow, goat or sheep milk (Appendix 12).

The fate of these pathogens during the manufacture of a raw milk Feta cheese was determined using a probabilistic model developed by the University of Tasmania and adapted by FSANZ.

Feta cheese is ripened under brine. Once coagulated the coagulum is cut and the curds are ladeled into moulds and left to drain until cohesion occurs. The cheese is then cut into pieces, salted and transferred to a brine solution for ripening. Generally Feta is stored at 2 - 4°C for at least 2 months. The manufacturing parameters and physicochemical properties for the modelled raw milk Feta cheese are based on experimental data and do not necessarily reflect

commercial manufacturing practices. These are provided in Table 34 and in Appendix 12 (Figure 1 and Table 1).

Table 34: Modelled raw milk Feta cheese manufacturing parameters and physicochemical properties

Step	Parameters/Properties
Acidification	pH <5.2 within 8.8 hours
1st Brine	12% solution for 2 hours (20 - 22°C)
Salt concentration after 1st brining	2.2%
2nd Brine	6% solution for 16 - 24 hours (16°C)
Salt concentration after 2nd brining	4.573%
Ripening period	4°C 90 days
Final pH	4.25 - 4.6
Final water activity	0.975
Final salt in moisture	4.6%

Key Findings

During the production of raw milk Feta it was estimated that the mean concentration of *E. coli* remained largely unchanged from those observed in the raw milk. For both *L. monocytogenes* and *S. aureus* there was a predicted increase in numbers with mean net concentration changes of 2.38 logand 1.13 log, respectively.

Using the qualitative framework, the risk from selected pathogens in this raw milk Feta cheese is characterised in Table 35.

Table 35: Risk characterisation of raw milk Feta cheese

Pathogen	Hazard characterisation	Exposure assessment	Risk Characterisation
E. coli (EHEC)*	High	Low	High
S. aureus	Negligible	Moderate	Low
L. monocytogenes	Negligible Moderate#	Moderate	Low High#

Susceptible populations

Conclusions

The process of manufacturing this raw milk Feta cheese results in no reduction of *E. coli* (EHEC) and therefore represents a **high** risk to consumers of both the general and susceptible population. The process of manufacturing raw milk Feta cheeses results in an increase in *L. monocytogenes* and therefore represents a **low** risk to the general population, however, for susceptible populations this risk is **high**. The process of manufacturing raw milk Feta cheese does not result in a reduction or increase of *S. aureus*, and represents a **low** risk to both general and susceptible populations While the risk from raw milk cheeses is based on a "per serve" basis it is estimated the likely consumption would be extremely low.

The process of manufacturing raw milk Feta cheese has been assessed to affect selected pathogens as describe in Table 36.

Table 36: Risk associated with raw milk Feta cheese

Pathogen	Risk associated with raw milk Feta cheese
E. coli (EHEC)	**High** risk as *E. coli* survives the cheesemaking process
S. aureus	Risk from *S. aureus* is considered **low**. Conditional on good control over animal health and raw milk handling.
L. monocytogenes	**Low** risk (general population) and **high** risk (susceptible population groups) as the organism survives and increases during the cheesemaking process.

There is no difference in the public health and safety risk from *L. monocytogenes* and *S. aureus* in raw milk Feta cheeses made from either cow, goat or sheep milk.

Due to the relatively low predicted inactivation of *E. coli* and the survival and possible increase of *L. monocytogenes* during the manufacture of raw milk Feta cheese, the microbiological quality of the raw milk and prevention of any contamination during manufacture are critical to microbiological safety.

Quantitative modelling indicates that in order to produce raw milk Feta cheese that would meet current microbiological limits in the Code, the initial concentration of *E. coli* and *L. monocytogenes* in the raw milk would need to less than 1 and 10^{-5} cfu/ml, respectively. In order to produce Feta cheese unlikely to contain sufficient staphylococcal toxin to cause illness (*i.e.* $<10^5$ cfu/g), the initial concentration in the milk would need to be less than 10^3 cfu/ml.

The findings of the modelled raw milk Feta cheese assessed cannot be applied to other semi-soft cheeses based on moisture (*i.e.* 43 - 55%) as the modelled cheeses does not represent all types of semi-soft cheeses in respect to physicochemical characteristics and manufacturing protocols. The findings however, may be applied to other Feta cheeses whose manufacturing specifications lie within the specification range of the modelled cheese.

10.6 Summary of raw milk Camembert cheese risk assessment

The qualitative framework was used to characterise the risk to public health and safety from *E. coli* (EHEC), *L. monocytogenes*, and *S. aureus* following the consumption of raw milk Camembert cheese made from either cow, goat or sheep milk (Appendix 13).

The fate of these pathogens during the manufacture of a raw milk Camembert cheese was determined using a probabilistic model developed by the University of Tasmania and adapted by FSANZ.

Camembert cheese is a soft cheese characterised by surface ripening using moulds such as *Penicillium Camemberti and Penicillium candidum*. Camembert cheeses are brine salted and initially ripened for 10-12 days at 12°C to enable mould formation, followed by storage at ~4°C for ~30 days.

The manufacturing parameters and physicochemical properties for the modelled raw milk Camembert cheese are based on experimental data and do not necessarily reflect commercial manufacturing practices. These are provided in Table 37 and in Appendix 13 (Figure 1 and Table 1).

Table 37: Modelled raw milk Camembert cheese manufacturing parameters and physicochemical properties

Step	Parameters/Properties
Acidification	<5.2 within 8.5 hours
Brine	2.25% for 15 - 75 minutes
1st Ripening period	12 - 13°C for 14 days
2nd Ripening period	4°C for 30 days
Final pH	7.2
Final water activity	0.98
Final salt in moisture	3.4%

Key findings

For raw milk Camembert cheese, the quantitative modelling predicted that there are no steps during production that result in an inactivation of the microorganisms investigated, leading to a substantial increase in microorganisms during cheesemaking.

Using the qualitative framework, the risk from selected pathogens in raw milk Camembert cheese is characterised in Table 38.

Table 38: Risk characterisation of raw milk Camembert cheese

Pathogen	Hazard characterisation	Exposure assessment	Risk Characterisation
E. coli (EHEC)	High	High	High
S. aureus	Negligible	High	Low
L. monocytogenes	Negligible Moderate#	High	Low High#

Susceptible populations

Conclusion

The results from the probabilistic modelling estimate that the process of manufacturing raw milk Camembert cheeses results in a substantial increase in *E. coli*, *L. monocytogenes*, and *S. aureus*. *E. coli* (EHEC) represents a **high** risk to general and susceptible populations. *L. monocytogenes* represents a **low** risk to the general population, however, for susceptible populations this risk is **high**. *S. aureus* represents a **low** risk to both general and susceptible populations. While the risk from raw milk cheeses is based on a "per serve" basis it is estimated the likely consumption would be extremely low.

The process of manufacturing raw milk Camembert cheese has been assessed to affect selected pathogens as described in Table 39.

Table 39: Risk associated with raw milk Camembert cheese

Pathogen	Risk associated with raw milk Camembert cheese
E. coli (EHEC)	**High** risk as the organism increases during cheesemaking and maturation.
S. aureus	Risk from *S. aureus* is considered **low**. Conditional on good control over animal health and raw milk handling. Substantial increase in levels during cheesemaking and maturation
L. monocytogenes	**Low** risk (general population) and **high** risk (susceptible population groups) as the organism increases both during cheesemaking and maturation

There is no difference in the public health and safety risk from *E. coli* (EHEC), *S. aureus* and *L. monocytogenes* in raw milk Camembert cheeses made from either cow, goat or sheep milk.

Due to the survival and possible increase of *E. coli* (EHEC), *L. monocytogenes* and *S. aureus* during the manufacture of raw milk Camembert cheese, the microbiological quality of the raw milk and prevention of any contamination during manufacture is critical to ensure microbiological safety.

Quantitative modelling indicates that in order to produce raw milk Camembert that would meet current microbiological limits in the Code, the initial concentration of *E. coli* and *L. monocytogenes* in the raw milk would need to less than 10^{-3} and 10^{-7} cfu/ml respectively. To produce Camembert cheese unlikely to have sufficient staphylococcal toxin to cause illness (*i.e.* $<10^{5}$ cfu/g), the initial concentration in the milk would need to be less than 10^{-4} cfu/ml.

It is possible the findings of the modelled raw milk Camembert cheese could be applied to the other mould ripened cheeses in the soft cheese category (*i.e.* >55% moisture). Soft mould ripened cheeses such as Camembert or similar type cheesesgenerally have similar physicochemical characteristics and manufacturing protocols *e.g.* minimal curd cooking, high moisture content and short ripening time. However, information pertaining to specific characteristics of the cheese (*e.g.* type of starter and adjunct cultures, type of mould etc) and manufacturing processes used would also need to be considered.

11. Discussion

The production of raw milk cheese represents less than 10% of total cheese production world-wide, however outbreaks of foodborne illness attributed to raw milk cheeses represents nearly 70% of all cheese attributed outbreaks.

Contamination of raw milk cheeses with *Salmonella* spp., *Brucella* spp., *L. monocytogenes* and *E. coli* are responsible for the majority of outbreaks in raw milk cheeses, with high moisture content (*e.g.* soft and fresh) cheeses those most often implicated.

Microbiological survey and monitoring data demonstrate that pathogens can be isolated from raw milk cheeses. Pathogens detected in raw milk cheeses internationally have included *Brucella* spp., *Mycobacterium bovis,* pathogenic *E. coli*, *L. monocytogenes*, *Salmonella* spp. and *S. aureus*.

While various *Brucella* spp. and *Mycobacterium bovis* have been detected and been responsible for a number of outbreaks of foodborne illness associated with the consumption of fresh raw milk cheeses, brucellosis in milk producing animals in Australia has been eradicated since 1989 and Australia has been recognised as bovine tuberculosis (*M. bovis*) free since 31 December 1997. However, contamination by *Brucella* spp. or *M. bovis* may pose a risk in imported raw milk cheeses.

The manufacture and ripening of cheese can be viewed as a complex interplay between several physical, biochemical and biological processes. Each process or condition of manufacture has some influence on the behaviour of bacteria in cheese. However, it is very difficult to determine the contribution of a single parameter to the growth, survival or death of any given pathogen. Instead a complex interaction between these parameters determines the potential for microorganisms to grow, survive or die in cheese and the combined effects of all parameters may often be greater than the sum of their effects.

Important intrinsic parameters include moisture content, pH and acidity, nutrient content, oxidation-reduction potential, presence of antimicrobial compounds, either those occurring naturally or those which are added as food preservatives such as nitrate, and the presence of competitive microflora. Extrinsic parameters include factors such as type of packaging/packaging atmosphere, time and temperature of storage and holding conditions, processing steps, product history and traditional use. All of these factors dictate the potential for bacterial pathogens to grow, persist or decline in cheeses.

Paramount to the safety of all raw milk cheeses is the microbiological quality of the raw milk.

Also critical for minimising the growth of pathogens in all raw milk cheeses is reaching the appropriate end point pH during acidification. Starter culture failure may result in pathogenic and spoilage microorganisms dominating the microbial population in the cheese leading to undesirable consequences such as the production of *Staphylococcus* enterotoxins that will remain in the cheese.

The role that curd cooking and ripening plays in ensuring the safety of raw milk cheeses differs according to the specific cheese type. The greatest lethal effect on pathogens is achieved through the application of heat, either to the raw milk or the cheese curd (*e.g.* pasteurisation, thermisation and curd cooking). The application of heat ranges from

being bacteriostatic in low curd cooked cheese to bacteriocidal for high curd cooked cheese. Pathogen die-off achieved during the ripening period is also extremely variable and depends upon the specific physicochemical characteristics of the cheese and the properties of the microorganism.

While there are many factors that influence the growth, survival and death of pathogens in cheeses, the factors during cheesemaking which have the greatest impact upon the microbiological safety of the raw milk cheeses evaluated include:

- The microbiological quality of the raw milk
- The acidification step
- The temperature and duration of curd cooking
- The temperature and duration of ripening

While cheesemaking involves several hurdles that influence the growth and/or survival of pathogenic microorganisms in cheese, it is a combination of hurdles, rather than an individual processing step or physicochemical properties that has the greatest impact on pathogen survival.

The extent to which each factor impacts upon the growth, survival and inactivation of microbiological hazards varies between individual cheeses, cheese types and manufacturing processes. Factors impacting on the raw milk cheeses assessed are described below.

Microbiological quality of raw milk

Raw milk is the major contributing source of microbiological contamination in raw milk cheeses, as the milk does not undergo an initial heat-treatment (*e.g.* pasteurisation or thermisation) which would eliminate or reduce the number of pathogen vegetative cells.

Campylobacter spp., *Salmonella* spp., *L. monocytogenes*, *S. aureus* and *E. coli* can contaminate raw milk either directly from the interior of the milking animal's udder of a diseased or infected animal, the exterior surfaces of the animals, the environment, milk-handling equipment, and personnel (Table 40).

Table 40: Sources of contamination from pathogens into raw milk

Microbiological hazard	Shed directly into milk	Exterior surface of animal *i.e.* faeces	Milking environment
Campylobacter spp.	✓	✓	x
E. coli (EHEC)	✓	✓	x
Salmonella spp.	✓	✓	✓
S. aureus	✓	x	✓
L. monocytogenes	✓	✓	✓

Primary production factors that impact upon the microbiological status of raw milk can be summarised as being:

- Animal-related factors *e.g.* animal health, herd size, age and production status
- Environment-related factors *e.g.* housing, faeces, feed, soil, and water
- Milking and operation of milking equipment factors *e.g.* cleanliness

Controlling the number of pathogens in raw milk therefore requires minimising contamination which arises from:

- Microorganisms being shed directly into raw milk from the udder as a result of disease or infection of the animal
- The external surface of the animal and the milking environment

The microbiological status of raw milk will be influenced by the extent to which producers control these primary production factors.

Modelling enabled predicted changes in pathogen levels to be estimated and allowed calculation of initial concentrations of *E. coli*, *L. monocytogenes*, and *S. aureus* required in the raw milk to produce raw milk Cheddar, blue, Feta and Camembert cheeses, that would meet the microbiological limits in the Code (Table 41). The Code specifies a limit of "less than 100 cfu/g" for *E. coli* in all cheeses and no *L. monocytogenes* and *Salmonella* spp. detectable in 25g for all raw milk cheeses.

Table 41: Initial concentration in raw milk required to meet current microbiological limits in the Code

Pathogen	Cheddar	blue	Feta	Camembert
E. coli	< 0.01 cfu/ml	n/a	<1 cfu/ml	$<10^{-3}$ cfu/ml
L. monocytogenes	$< 10^{-3}$	$<10^{-5}$	$<10^{-5}$ cfu/ml	$<10^{-7}$ cfu/ml
*S. aureus**	<100 cfu/ml	n/a	$<10^{3}$ cfu/ml	$<10^{-4}$ cfu/ml

* Initial numbers to ensure numbers do not reach levels that may produce enterotoxin to cause illness (*i.e.* $<10^{5}$ cfu/g) as there is no limit for *S. aureus* in the Code.

Acidification

The production of acid at the appropriate rate and time is critical for the cheesemaking process and to ensure the microbiological safety of the final cheese. Acid production and the resultant decrease in pH affects the growth of many non-starter bacteria, including pathogens which may be present in the raw milk. During the first 24 hours (including the early stages of ripening) the production of lactic acid by the starter culture is important in limiting the growth of pathogenic bacteria. Most pathogenic bacteria require a neutral pH for optimum growth and grow poorly at pH values below 5.0. A pH drop to 5.2 - 5.5 during the first 24 hours occurs in most cheese varieties.

Curd cooking temperature

Of all factors that control microorganisms in cheese, the heat treatment applied to raw milk or to the cheese curd (curd cooking) has the greatest impact upon the survival and growth of microbial pathogens. As raw milk cheeses do not undergo any initial heat-treatment (*e.g.* pasteurisation), curd-cooking at elevated temperatures has the greatest effect on reducing numbers of pathogens that may be present in the curd.

The maximum temperature for growth for most pathogens is 45 - 48°C, therefore curd cooking at temperatures 48°C and above will begin to have a lethal effect. Curd cooking at temperatures in excess of 55°C for periods greater than 40 minutes, such as in the manufacture of some extra hard and Swiss-type cheeses, is sufficient to significantly decrease the numbers of pathogens that may be present in raw milk.

Long ripening

During maturation the combined effects of low pH, high salt, reduced moisture and ripening temperature come into play and promote the die-off of various pathogens.

The conditions during maturation tend to see a combination of intrinsic properties and environmental conditions which are sub-optimal for pathogenic bacteria. While individually they have little impact, in combination these hurdles result in bacterial die-off over a prolonged period of ripening.

Most bacteria require a minimum water activity of around 0.92 for growth. Cheeses that have been ripening for a long period of time will typically have a low water activity ***i.e.*** less than 0.92, and this inhibits the growth of most pathogens. Conversely, cheeses which have a short ripening period will have a higher water activity, and may support the growth of pathogens.

Holding cheese at elevated temperatures promotes faster ripening and faster loss of moisture, and therefore results in greater die-off of bacterial pathogens, particularly *E. coli*.

In assessing the safety of raw milk cheeses, risk assessments for raw milk extra hard, Swiss-type, Cheddar, blue, Camembert, and Feta style cheeses were undertaken to assess the risk to public health and safety presented by selected microbiological hazards following consumption of these cheeses.

Quantitative modelling developed by the University of Tasmania and adapted by FSANZ was used to determine the fate of pathogens for raw milk Cheddar, blue, Feta and Camembert cheese. The manufacturing parameters and physicochemical properties for the modelled raw milk cheeses, however, are based on experimental data and do not necessarily reflect commercial manufacturing practices.

A summary of the net change in the predicted modelled growth or inactivation of *E. coli, S. aureus* and *L. monocytogenes* in raw milk Cheddar, blue, Feta and Camembert cheeses is illustrated in Figure 9.

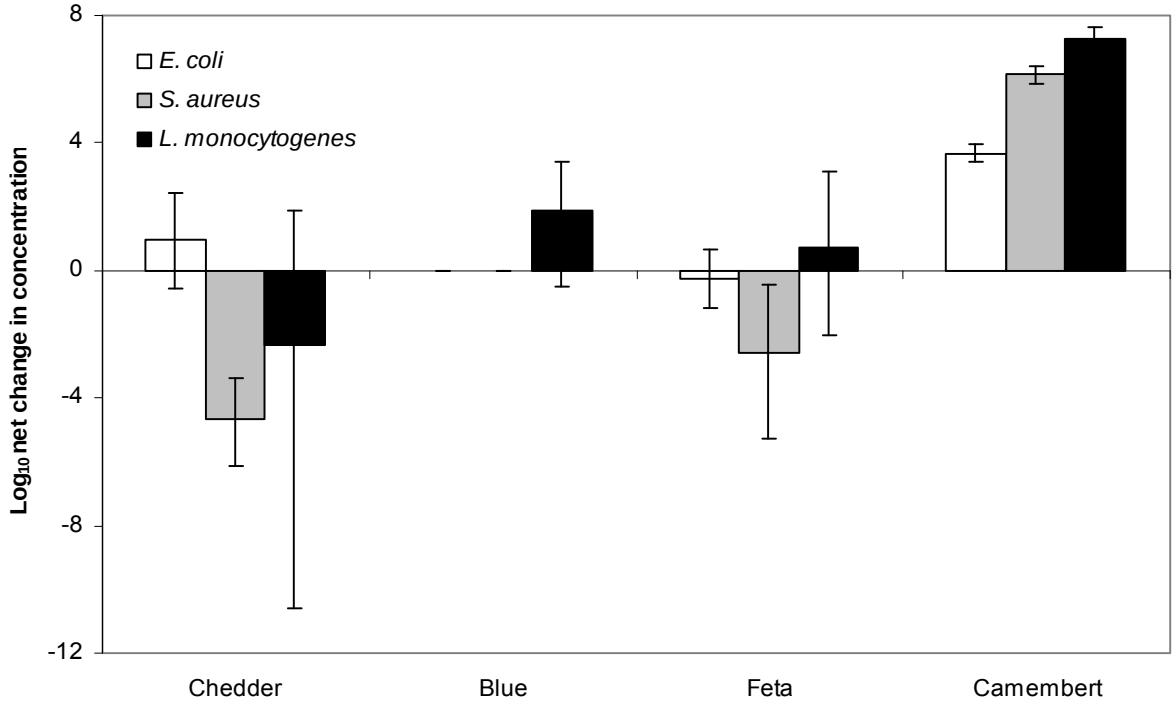


Figure 9: Net change (log10 cfu/g) in the predicted concentration of *E. coli, S. aureus* and *L. monocytogenes* in raw milk Cheddar, blue, Feta and Camembert cheeses. The bars are the predicted mean change. The error bars indicate the 5th and 95th percentile values.

The findings of the quantitative modelling are consistent with results from published challenge studies. The development of the quantitative models has incorporated both variability and uncertainty in the behaviour of pathogens during cheese production and ripening/maturation as well as cheese production processing and storage conditions. In particular there is large variability between the surivival of various strains of the same pathogen; this is particularly evident in the survival of *L. monocytogenes* in Cheddar cheese.

Variability between challenge studies can result from the selection of appropriate pathogens or surrogates; the level of challenge inoculum; the inoculum preparation and method of inoculation; the duration of the study; formulation factors and storage conditions; and sample analyses. In addition, challenge studies which been based on the use of pasteurised or UHT milk rather than raw milk may overestimate survival of pathogens during ripening and maturation due to a lack of competitive microflora.

Rates of pathogen inactivation during ripening/maturation in the quantitative modelling were similar in comparison to results from published challenge studies; however there was high variability between strains, particularly in *L. monocytogenes* (illustrated in the error bars in Figure 9). The predicted growth during the initial phase of cheesemaking, however, was higher in the model than the challenge studies.

The inclusion of a lag phase in the model and the inhibition of growth due to rapid pH decline during acidification would reduce this difference between the reported studies and the model predictions. However, the inclusion of a lag phase is only likely to reduce the predicted growth of microorganisms during the initial phase of cheesemaking by approximately 1 log. This is assuming a lag phase of approximately 2 hours. Consideration of temporal changes in

the physicochemical characteristics and their effect on survival prior to ripening would be more significant and would affect the final concentration in the cheese at the end of production.

For raw milk Cheddar cheese, the results of the quantitative modelling indicate minimal reduction of *E. coli* during maturation. The inactivation of *L. monocytogenes* during maturation was found to be highly variable depending upon the strain. Nevertheless survival of some strains is likely. These findings are consistent with challenge studies for *E. coli* (Schlesser *et al.,* 2006: Reitsma and Henning, 1996: Teo *et al.,* 2000) and *L. monocytogenes* (Ryser and Marth 1987b: Reistma and Henning, 1996: Ryser and Marth, 1999).

Growth and/or survival of *E. coli*, *S. aureus* and *L. monocytogenes* in Feta cheese has been demonstrated in various challenge studies (Ramsaran *et al.,* 1998: Govaris *et al.,* 2002: Manolopoulou *et al.,* 2003: Papageorgiou and Marth, 1989a). These results are consistent with the results of the quantitative modelling, although a greater net log change was observed in the model due to greater inactivation during ripening.

The quantitative modelling indicates that raw milk Camembert supports the growth of *E. coli*, *S. aureus* and *L. monocytogenes.* These results are consistent with the findings of other challenge studies (Ramsaran *et al.,* 1998: Matsusaki *et al.,* 1991: Ryser and Marth, 1987a: Back *et al.,* 1993).

Survival and/or growth of *L. monocytogenes* in raw milk blue cheese is supported by the findings of the quantitative modelling and challenge studies (Papageorgiou and Marth, 1989b).

The risk to public health and safety presented by selected microbiological hazards from the consumption of raw milk extra hard, Swiss-type, Cheddar, blue, Camembert, and Feta style cheeses using a qualitative framework is summarised in Table 42.

Table 42: Principal risks to public health and safety from selected raw milk cheeses

Hazard	Extra Hard	Swiss	Cheddar	Blue	Feta	Camembert
C. jejuni	Negligible	Negligible				
E. coli (EHEC)	Low	Low	High		High	High
Salmonella spp.	Negligible	Negligible				
S. aureus	Negligible	Negligible	Very low		Low	Low
L. monocytogenes	Negligible Very low[#]	Negligible Very low[#] Low/High[1,#]	Negligible Low[#,2]	Low High[#]	Low High[#]	Low High[#]

\# Susceptible populations
1 Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine
2 Sheep milk

Campylobacter spp. were found to be a **negligible** risk in both raw milk extra hard and Swiss-type cheeses. The presence of *Campylobacter* spp. was not assessed in raw milk Cheddar, blue, Feta or Camembert cheeses. However, *Campylobacter* spp. are unlikely to grow in milk or cheese, as their growth requires reduced oxygen tension and temperatures between 32 - 45°C and they do not survive well under slightly acidic conditions, or in the presence of greater than 2% salt.

E. coli (EHEC) was found to be a **low** risk in raw milk extra hard and Swiss-type cheeses, a **high** risk in Cheddar, Feta and Camembert cheese. The fate of *E. coli* (EHEC) was not assessed in raw milk blue cheese due to a lack of data. In general, *E. coli* numbers increase during the manufacture of cheeses (up to 3 log increase), with counts decreasing during ripening. However, this is highly variable between cheeses.

Salmonella spp. were found to be a **negligible** risk in both raw milk Swiss and extra hard cheeses. The fate of *Salmonella* spp. in raw milk Cheddar, blue, Feta and Camembert cheeses was not assessed; however *Salmonella* spp. counts increase initially during cheese manufacture before generally decreasing during maturation/ripening. As a member of the *Enterobacteriaceae* family, *Salmonella* spp. have many of the survival and growth characteristics of *E. coli*.

S. aureus was found to be a **negligible** to **low** risk in all raw milk cheeses. However this is dependent upon low initial levels in the raw milk and the prevention of growth and enterotoxin production during fermentation. High numbers of staphylococci ($>10^5$ cfu/ml) are required for the production of sufficient heat stable enterotoxins to cause illness. Raw milk that is not cooled rapidly or stored correctly will support growth and possible toxin production. *S. aureus* will also be a concern if fermentation fails. Rapid pH fall is critical for restricting pathogen growth and toxin production in cheese during the early stages of production.

L. monocytogenes was found to be a **negligible** to **low** risk for the general population for all raw milk cheeses assessed. However, for susceptible populations *L. monocytogenes* was found to be a **high** risk in raw milk Swiss Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine cheeses; blue; Camembert and Feta cheeses. Generally *Listeria* counts initially increase during cheese manufacture, while its decrease during maturation/ripening is variable between cheeses and also within the same cheese type. The variability in decline during maturation is reflected in the challenge studies used to develop the predictive models. *L. monocytogenes* can grow at low temperatures and across a wide pH range. It can also grow in salt concentrations of up to 10 - 14% and tolerates low water activity. These tolerances enable this organism to survive in many cheese types and environments.

There is no difference in the public health and safety risk from the pathogens assessed in raw milk extra hard, Swiss-type, blue and Camembert cheeses made from either cow, goat or sheep milk. However, in raw milk Cheddar cheeses, *L. monocytogenes* presents a greater risk when produced from raw sheep milk compared to the same cheese produced from cow or goat milk. This is a result of both *L. monocytogenes* and *E. coli* having a greater prevalence in raw sheep milk compared to cow and goat milks. Changes in the prevalence of microbiological hazards in raw milk can impact on the estimated level of risk.

Consumption data on raw milk cheeses is unavailable in Australia. Cheese production statistics show that hard and semi-hard cheeses account for 75% of Australia's cheese production, whereas soft and blue style cheeses account for less than 1%. Data from the NNS indicates that consumption of extra hard, Swiss-type, blue, Feta and Camembert/Brie cheeses is extremely low whereas Cheddar cheese is the most commonly consumed cheese (26.5% of those surveyed) in Australia. This data reflects historical trends, as Australians are now eating a much wider variety of specialty cheeses, and there appears to be a growing demand for these cheeses, including interest in raw milk cheeses.

The ability to apply the findings of the specific cheeses evaluated, to assess the safety of other cheeses within the same moisture category, was variable and is summarised in Table 43. While the cheeses assessed are examples of very hard, hard, semi-soft and soft cheese based on moisture content they are not necessarily representative of all cheeses found in these categories. For example, the modelled blue cheese may be considered a semi-soft cheese when classified on moisture content, but not all semi-soft cheeses are mould ripened *e.g.* Brick, Edam and Gouda. In addition, subdivision of cheeses based on moisture can be arbitrary and overlapping. While cheeses may be often grouped together on moisture content, these cheeses may widely differ in physicochemical characteristics and manufacturing protocols *e.g.* Cheddar, Parmesan and Emmentaler are often grouped together as hard cheeses. Factors impacting on the raw milk cheeses assessed are summarised in Table 44.

The significant lack of suitable data, and variability in data which is available, emphasises the difficulty of evaluating the safety of raw milk cheeses. Assessment of the safety of raw milk cheese requires:

- Detailed information and data on the individual cheesemaking processes
- Details of the physicochemical properties of the final cheese
- Challenge study data

Table 43: Comparison of risk assessment findings to cheese types

Raw milk cheese assessed	Moisture category	Findings applicable to moisture category	Findings applicable to cheese type	Comments
Parmigiano Reggiano Grana Padano Romano Asiago Montasio Sbrinz	Extra hard (<36%)	Applicable	Applicable	The cheeses assessed are likely to represent other cheeses in the extra hard moisture category. Extra hard cheeses generally have similar physicochemical characterises and manufacturing protocols *e.g.* curd cooking and long ripening times.
Emmentaler Gruyère Appenzeller Tilsiter Vacherin Fribourgeois Tête de Moine	Hard (37 - 42%)	Not Applicable	Applicable (Internal bacterially ripened cheese with eyes - lactate)	Moisture contents of assessed Swiss-type cheeses overlap between the extra hard and hard moisture categories (31 - 44%) and are not representative of all hard cheeses. This group of bacterially ripened cheeses with eyes has different physicochemical characteristics and manufacturing protocols to other hard and extra hard cheeses.
Cheddar	Hard (37 - 42%)	Not Applicable	Applicable (Internal bacterially ripened hard cheese)*	Cheddar is a milled, dry-salted cheese having different physicochemical characteristics and manufacturing protocols to other hard cheese, and therefore does not represent all hard cheese.
Blue	Semi-soft (43 - 55%)	Not Applicable	Applicable (Mould ripened –internal mould cheese)*	Moisture contents of blue cheeses vary and can overlap between moisture categories from soft to semi-soft/semi-hard. The physicochemical characteristics of other cheeses within the mould ripened (internal mould) category are also variable.
Feta	Semi-soft (43 - 55%)	Not Applicable	Applicable (Internal bacterially ripened high salt variety)*	Feta cheese is not representative of all semi-soft cheeses. This high salt variety has very different physicochemical characteristics and manufacturing protocols to other semi-soft cheeses.
Camembert	Soft (>55%)	Applicable	Applicable (Mould ripened – surface mould cheese)*	Camembert cheese is likely to represent other cheeses in this same moisture category as cheeses in this category generally have similar physicochemical characteristics and manufacturing protocols *e.g.* minimal curd cooking, high moisture content,and short ripening times.

* Cheeses whose manufacturing parameters lie within the range of those of the modelled raw milk cheese

Table 44 Summary of factors affecting raw milk cheese during cheesemaking

Raw milk cheese	Factors of greatest impact on safety of cheese during cheesemaking	Uncertainty/variability
Extra hard Parmigiano Reggiano, Grana Padano, Romano, Asiago, Montasio Sbrinz	High curd cook cheeses – *e.g.* Parmigiano Reggiano, Grana Padano and Sbrinz • Microbiological quality of raw milk • Acidification • Curd cooking Low curd cook cheeses – *e.g.* Pecorino Romano, Asiago, Montasio • Microbiological quality of raw milk • Acidification • Maturation	
Swiss-type Gruyere, Emmentaler Appenzeller, Tilsiter, Tête de Moine, Vacherin Fribougeois	Gruyere, Emmentaler • Microbiological quality of raw milk • Rapid acidification • Curd cooking Appenzeller, Tilsiter, Tête de Moine, Vacherin Fribougeois • Microbiological quality of raw milk • Acidification • Maturation	Lack of data about the fate of pathogens in the Swiss-type cheeses: Appenzeller, Tête de Moine, and Vacherin Fribougeois. Therefore the safety of these cheeses is based on the likely effect of cheesemaking stages. The physicochemical properties and manufacturing processes between Swiss-type cheeses assessed are variable.
Cheddar	• Microbiological quality of raw milk • Acidification	While there is die-off of *E. coli* (EHEC) during maturation in Cheddar cheese, there is considerable variability about the extent of this reported in challenge studies. The physicochemical properties and manufacturing processes reported for Cheddar cheese are highly variable.
Blue	• Microbiological quality of raw milk • Acidification	Uncertainty about the fate of *L. monocytogenes* in blue cheese with differing results reported in challenge studies and probabilistic model. The physicochemical properties and manufacturing processes reported for blue cheese are extremely variable. Significant lack of data on the fate of other pathogens in various blue cheeses.
Feta	• Microbiological quality of raw milk • Acidification	Considerable variability about the fate of *E. coli* and *L. monocytogenes* in Feta cheese with differing results reported in challenge studies and probabilistic model. The physicochemical properties and manufacturing processes reported for Feta cheese are extremely variable.
Camembert	• Microbiological quality of raw milk • Acidification	Slight variability about the fate of *L. monocytogenes* in Camembert cheese with differing results reported in challenge studies and probabilistic model. The physicochemical properties and manufacturing processes reported for Camembert cheese are extremely variable.

12. Data gaps and research needs

A number of data gaps were identified in the risk assessment for which assumptions were made. Further information/research into these data gaps will assist in reducing the amount of uncertainty in the levels of estimated risk for the various raw milk cheeses assessed.

12.1 Incidence and prevalence data for pathogens in raw milk

There is limited published data on the prevalence and levels of pathogens in raw milk both within Australia and overseas. Within Australia, there is no regular or ongoing surveillance of pathogenic microorganisms in raw cow and sheep milk. There is some surveillance of raw goat milk in a few States where its production and sale are permitted. Surveillance of raw milk does not include quantification, including levels and typing.

Further information on the incidence and prevalence of pathogens in raw milk would reduce uncertainty in the final estimation of risk. Such quantitative data would facilitate the development of models to determine the fate of specific organisms in various cheese types.

12.2 Cheesemaking process

There is limited information on individual cheesemaking processes (including the physicochemical characteristics/properties of individual cheeses). While generic processes for various cheese types are reported in the literature, individual cheesemaking processes may vary and will impact on the inactivation, survival and growth of pathogenic microorganisms.

Further data on cheesemaking processes would assist in determining similarities between cheeses, and whether the findings of an assessment can be applied to other cheese varieties.

12.3 Physicochemical characteristics/properties of specific cheeses

There is limited information on physicochemical characteristics of individual cheeses and on the effect of changing physicochemical characteristics during cheese maturation on pathogens. Consequently the outputs of the probabilistic models were unable to be applied to other cheeses of varying characteristics. Varying physicochemical characteristics of individual cheeses will have a variable impact upon the inactivation, survival or growth of pathogens in raw milk cheese.

Further information would allow the modelling approach to be broadened to consider the effect of changing physicochemical properties (*e.g.* pH, water activity, temperature and time) throughout the cheesemaking process, provided there was also sufficient data on the inactivation kinetics.

12.4 Data on survival, inactivation and growth of pathogenic microorganisms during the cheesemaking process

There is a significant lack of data on the survival, inactivation and growth of various pathogens in raw milk cheese and between different strains of the same organism. Data where available is limited and very cheese specific. In particular, for this risk assessment, there was insufficient data to model the fate of *E. coli* and *S. aureus* in blue cheese and

therefore further data on the inactivation of these two microorganisms would enable an assessment of blue cheese.

12.4.1 Strain variation

There is limited data on the inactivation of different strains of *E. coli* and *S. aureus*. There is also a large variation in the inactivation rates reported between strains of *L. monocytogenes* during the ripening of various cheeses.

Further information on the strains most likely to be encountered in a cheese production facility would further reduce the overall uncertainty on the inactivation kinetics during ripening for *L. monocytogenes*. Whereas incorporating different strains in the model for *E. coli* and *S. aureus* would increase the overall variability in the output; but would also reduce the level of uncertainty.

12.4.2 Growth/no growth boundaries

There is a lack of data on the effect of a rapid decline in pH on the inactivation of pathogens during acidification by the starter culture. Similarly, there is a lack of data on the growth/inactivation boundaries of salt during cheesemaking.

Further information, specifically on the temporal changes in the physicochemical characteristics and their effects would enable the model to consider growth/no growth boundaries and provide more realistic risk estimates.

12.4.3 Effect of lactic acid during fermentation on growth of pathogens

There is little information regarding the development of lactic acid during fermentation of specific cheeses and in particular there are no known growth rate models available that describe the effect of lactic acid on *S. aureus*.

Further information on the development of lactic acid and growth models for *S. aureus* would allow the explicit inclusion of the effect of lactic acid in the probabilistic model which could reduce final concentration of the *S. aureus* in all cheeses modelled.

12.4.4 Effect of competitive microflora in raw milk cheeses

There is very limited data on the effect of competitive microflora in the raw milk cheeses modelled. Inclusion of data on the effect of competition on the growth of the three pathogens would give greater accuracy to initial growth rate estimates and ultimately the final concentration of the organisms in the cheese.

12.4.5 Effect of lag phase on growth during cheese manufacture

There is a lack of data on the length of the lag phase for microorganisms during the initial phase of cheese manufacture. Further information on lag times during cheesemaking may allow the addition of a lag phase in the probabilistic model and may reduce the predicted growth of microorganisms during the initial phase of cheese manufacture. However, this is not likely to be as significant as including growth/no growth boundaries in the model.

12.5 Consumption data

Data on the frequency and amount of raw milk cheese consumed in Australia is extremely limited, as permissions are in place for only a few cheese types. There is no information on the likely consumption or demographics of consumers who would possibly consume raw milk cheese if permitted.

Further quantitative and/or qualitative data on likely consumption would assist in contextualising the risk of illness resulting from consumption of raw milk cheese, if permitted.

12.6 Extent and cause of sporadic human cases of raw milk cheeses associated foodborne illness

Outbreak data is not necessarily indicative of the true incidence and causes of sporadic raw milk cheese associated foodborne illness. Attribution of sporadic cases is difficult due to factors such as the general under-reporting of foodborne illness, retrospective nature of foodborne illness investigation, the often non-point source nature of exposure and the low frequency of consumption.

13. Conclusions

The diversity of cheese is immense, with hundreds of varieties of cheese produced all over the world. Different varieties of cheese are the result of using different species of bacteria and moulds, different levels of milk fat, variations in length of ripening, differing processing treatments (cheddaring, pulling, brining, mould/surface treatment washes) and use of milk from different breeds of cows, goats, sheep, or other mammals - all resulting in infinite variations in the physicochemical properties of the final cheese.

While cheese has been produced for centuries using raw milk, the advent of pasteurisation in the 20th century had an important role in enhancing the safety of many cheeses. However, outbreaks of foodborne illness attributed to cheese, and in particular raw milk cheese, continue to be reported internationally.

Salmonella spp., *L. monocytogenes, S. aureus* and *E. coli* (EHEC) pose the greatest risk to the safety of both pasteurised and raw milk cheeses and this is confirmed by the number of outbreaks associated with these organisms in cheeses.

Nevertheless, a range of raw milk cheeses continue to be manufactured internationally, relying upon hurdles such as rapid acidification, cooking steps, low water activity and prolonged ripening which can, in certain circumstances, provide protection against the presence and/or proliferation of pathogenic microorganisms.

Data on the consumption of raw milk cheeses within Australia is not available. However, data from Australian food production statistics and the 1995 National Nutrition Survey (NNS) show consumption of extra hard, Swiss-type, blue, Feta and Camembert cheeses, and speciality cheeses in general, is very low. It is therefore likely that the potential consumption of raw milk cheeses would also be very low.

The risk assessment demonstrates that raw milk cheese may be contaminated with a range of pathogenic microorganisms.

Probabilistic modelling demonstrated that the overall effect of cheesemaking on pathogens was:

- *E. coli* was able to survive and grow in Cheddar, Feta and Camembert cheeses
- *L. monocytogenes* was inactivated during maturation in Cheddar cheese, however, this inactivation was variable and some strains survived. The modelling also demonstrated that *L. monocytogenes* was able grow in blue, Feta and Camembert cheeses
- *S. aureus* could grow in Feta and Camembert cheese. There was insufficient data available to model the fate of *E. coli* or *S. aureus* in blue cheese

Using a qualitative framework, the risk assessment concluded that the risk posed by *C. jejuni/coli*, *E. coli* (EHEC), *Salmonella* spp., *S. aureus* and *L. monocytogenes*, from consumption of raw milk extra hard and Swiss-type cheeses is **low** to **negligible** in the general population. *L. monocytogenes* was however rated **high** for raw milk Swiss Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine cheeses in susceptible populations.

Using the qualitative framework the principal risks to public health and safety from the consumption of raw milk Cheddar, blue, Feta and Camembert cheeses are:

- *E. coli* (EHEC) was rated **high** risk in raw milk Cheddar, Feta and Camembert cheeses
- *L. monocytogenes* was rated **high** risk for susceptible populations in blue, Feta and Camembert cheese

Quantitative modelling indicates that in order to produce raw milk Cheddar, blue, Feta and Camembert cheeses that would meet the current microbiological limits for *E. coli, L. monocytogenes,* and *S. aureus* in the Code, the initial concentration in the raw milk would need to be extremely low (*i.e.* for raw milk Camembert cheese the initial concentration of *E. coli* and *L. monocytogenes* in the raw milk would need to be less than 10^{-3} and 10^{-7} cfu/ml, respectively to meet microbiological limits in the Food Standards Code).

The factors during cheesemaking that contribute to the microbiological safety of cheese include milk quality, extent of acidification by the starter culture, the amount of heat applied at various stages during the manufacture of cheese, pH, salt content, and reduced water availability resulting from salting and ripening. Pathogens will grow more easily in cheese of high moisture content, neutral pH and low salt content, compared to the more hostile environment of high temperature curd cooked cheeses ripened over a prolonged period *e.g.* extra-hard cheese.

As raw milk cheeses do not undergo a pathogen elimination step such as pasteurisation, those factors that have a significant impact upon the microbiological safety of the cheeses are the microbiological quality of the raw milk, the acidification step, the temperature and duration of curd cooking, and the temperature and duration of ripening. However, the extent that these factors impact upon the growth, survival and death of microbiological hazards varies significantly between individual cheeses as the inherent characteristics and processing parameters between cheeses and cheese types vary considerably. It must also be stressed that while each of these factors individually has an effect, it is their combined effect that has the greatest impact on the growth or survival of pathogens in cheese.

While the cheeses selected are examples of very hard, hard, semi-soft and soft cheese based on moisture content they are not representative of all cheeses found in these moisture categories. For example, the modelled blue cheese may be considered a semi-soft cheese when classified on moisture content, but not all semi-soft cheeses are mould ripened *e.g.* Brick, Edam and Gouda. In addition, subdivision of cheeses based on moisture can be arbitrary and overlapping. While cheeses may be often grouped together on moisture content, these cheeses may widely differ in physicochemical characteristics and manufacturing protocols *e.g.* Cheddar, Parmesan and Emmentaler are often grouped together as hard cheeses.

The ability to apply the findings of the specific cheeses evaluated to other cheeses within the same moisture category was variable.

The risk assessment highlighted the difficulty in evaluating the safety of raw milk cheeses due to the lack of suitable data, as well as the variability of the data which is available. Variability and uncertainty have been included where possible in the evaluation to assess the fate of pathogens during cheese production. However, safety assessments of raw milk cheese

require detailed information on the specific manufacturing process, physicochemical characteristics of the cheese and challenge data.

In assessing and seeking to manage the safety of raw milk cheeses it is important to reflect on the complexities associated with exercising control over the cheesemaking process. The initial fermentation phase of cheesemaking sees the setting and coagulation of milk proteins to produce a curd which the cheesemaker then manipulates to produce a matrix which, when ripened, bears the specific characteristics of that type of cheese.

In large sophisticated cheesemaking operations, many of the processes are highly mechanised and automatically controlled. However in small, artisinal cheesemaking operations, the art of speciality cheesemaking still prevails, resulting in considerable heterogeneity in the outputs.

Cheese texture, flavour and aroma are the result of careful control over the initial fermentation process (acid production, synerisis, salting and curd handling) to produce a substrate that when ripened is cheese. The properties of the coagulum are influenced by seasonal variations in milk (especially protein content), the pH of coagulation and the level of calcium in the milk, and by the way the cheesemaker manages setting, moisture retention, acid production and the cooking processes. Not surprisingly, cheeses of the same type have varying physicochemical properties and their safety is very much influenced by the way the cheesemaker manages the initial phase of cheesemaking.

APPENDICES

APPENDIX 1: Qualitative framework for categorising hazards

1 Matrix

Colour code

- High
- Moderate
- Low
- Very Low
- Negligible

Hazard characterisation
(Severity of Hazard)

	Consequences of exposure			
"Infective dose"	Mild	Moderate	Serious	Severe
<10				
10 -100				
100 - 1,000				
>1,000				

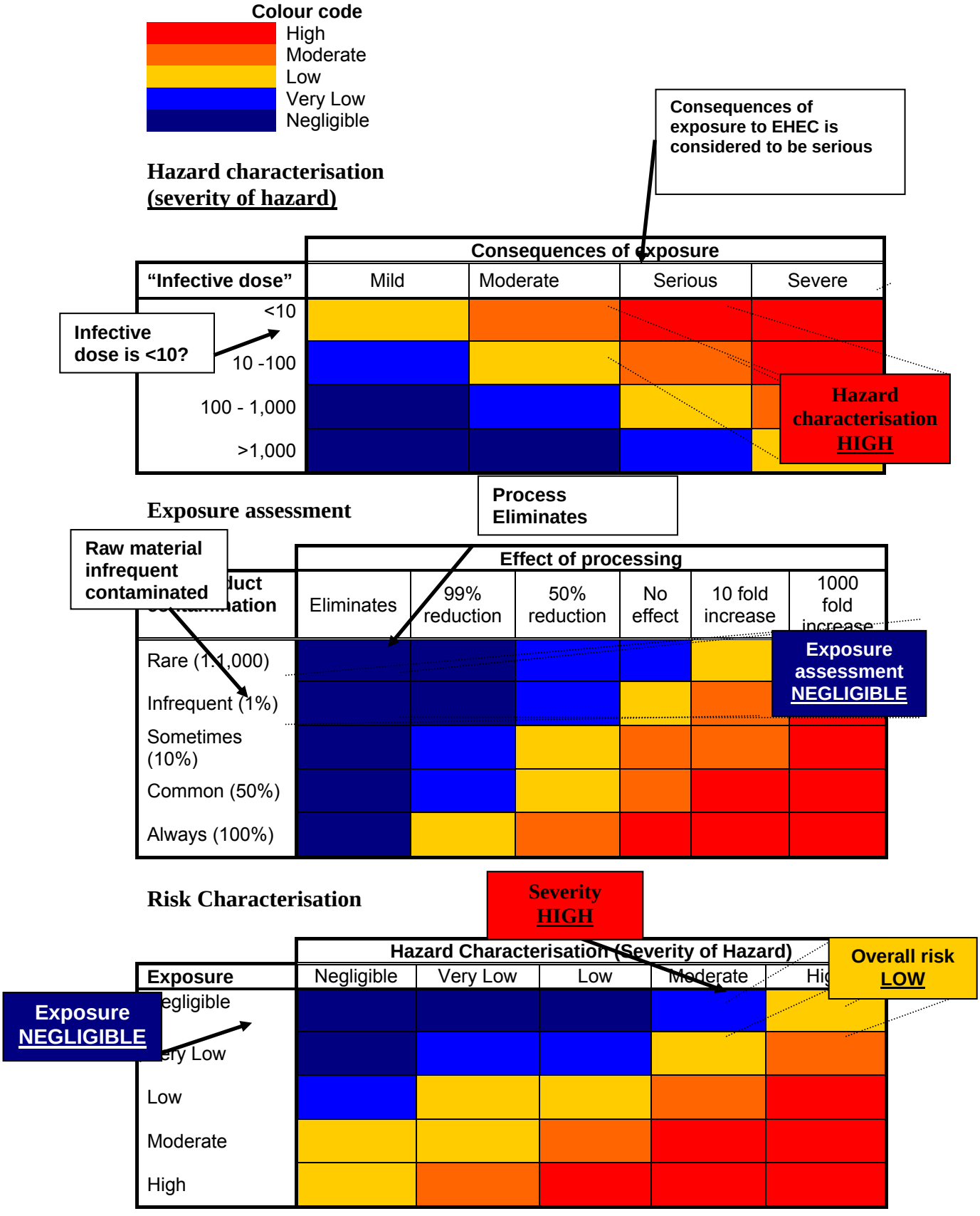
Exposure assessment

	Effect of processing					
Raw product contamination	Eliminates	99% reduction	50% reduction	No effect	10 fold increase	1000 fold increase
Rare (1:1,000)						
Infrequent (1%)						
Sometimes (10%)						
Common (50%)						
Always (100%)						

Risk Characterisation

	Hazard Characterisation (Severity of Hazard)				
Exposure	Negligible	Very Low	Low	Moderate	High
Negligible					
Very Low					
Low					
Moderate					
High					

Figure 1: Example of risk categorisation of EHEC in extra hard (high temperature curd cook) raw milk cheese for the general population



2 Assumptions used in determining risk

Table 1: Hazard characterisation and consequences of exposure

Hazard Characterisation			
Hazard	**Infective dose**	**Consequences of exposure**	
		General	**Susceptible**
Campylobacter jejuni	100-1,000	Moderate	Serious
***Escherichia coli* (EHEC)**	<10	Serious	Serious
Listeria monocytogenes	>1,000 (10-100)[#]	Moderate	Severe
***Salmonella* spp.**	10-100	Moderate	Serious
Staphylococcus aureus	>1,000	Mild	Mild

[#] Susceptible populations

Table 2: Consequences of exposure determinations

Organism	Severity of illness (ICMSF)		Consequences of exposure[1] (Qualitative Framework)	
	General population	**Susceptible**	**General population**	**Susceptible**
C. jejuni/coli	-	Severe	Moderate	Serious
***E. coli* (EHEC)**	Severe		Serious	Serious
L. monocytogenes	Serious	Severe	Moderate	Severe
***Salmonella* spp (non typhi)**	Serious		Moderate	Serious
S.aureus	Moderate		Mild	Mild

[1] Refer to Definitions Table for definitions

The qualitative framework was developed by Food Science Australia. It employs elements of Risk Ranger (Ross and Sumner, 2002) as well as uses ICMSF (ICMSF, 2002) classifications for judging the severity of foodborne illness caused by selected pathogenic organisms. The descriptors used in the framework are an amalgam of information from these sources combined with expert elicitation using members of the Dairy Scientific Advsiory Panel and information from epidemiological investigations.

Table 3: Definitions used for consequence of exposure determinations

ICMSF	Risk Ranger	Qualitative Framework
SEVERE Life threatening, or substantial sequelae, or long duration	**SEVERE** Causes death to most victims	**SEVERE** – Life threatening, with substantial sequelae, or long duration, Causes death to many victims, with a case fatality rate of >10%
SERIOUS Incapacitating but not life threatening; sequelae infrequent; moderate duration	**MODERATE** Requires medical intervention in most cases	**SERIOUS** – Incapacitating and potentially life threatening, with or without substantial sequelae, or long duration. Requires medical intervention in >20% of cases
MODERATE Not usually life threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort	**MILD** Sometimes requires medical intervention	**MODERATE** - Incapacitating but not life threatening, sequelae infrequent and of moderate duration. <20% of cases require medical attention

Table 4: Contamination of Australian raw milk

Organism	Raw product contamination		
	Cow	Goat	Sheep
C. jejuni	Infrequent (1%)	Infrequent (1%)	Rare (1:1000)
E. coli (EHEC)	Infrequent (1%)	Infrequent (1%)	Sometimes (10%)
L. monocytogenes	Infrequent (1%)	Infrequent (1%)	Sometimes (10%)
Salmonella spp.	Infrequent (1%)	Infrequent (1%)	Rare (1:1000)
S. aureus	Sometimes (10%)	Common (50%)	Sometimes (10%)

Table 5: Justification for contamination of raw cow milk

Organism	Raw product contamination	Justification
C.r jejuni	Infrequent (1%)	Australian data 0%,International data 0 – 40%. Expert panel consultation.
E. coli (EHEC)	Infrequent (1%)	Australian data 1 – 3%. International data 0 – 33.5%. Expert panel consultation.
L. monocytogenes	Infrequent (1%)	Australian data 0%, International data 1 – 60%. Expert panel consultation.
Salmonella spp.	Infrequent (1%)	Australian data 6.2%, International data 0 – 11.8%. Expert panel consultation.
S. aureus	Sometimes (10%)	Australian data 22.9% (CP Staph), International data 9.7 – 100%. Expert panel consultation.

Table 6: Justification for contamination of raw goat milk

Organism	Raw product contamination	Justification
C. jejuni	Infrequent (1%)	Australian data 1.39%, International data 0 – 0.04%. Expert panel consultation.
E. coli (EHEC)	Infrequent (1%) (Assumed from *E. coli* data)	Australian data 7.37% (E. coli), International data 0 – 16.3%. Expert panel consultation.
L. monocytogenes	Infrequent (1%)	Australian data 0-6.8 %, International data 0 – 5.8%. Expert panel consultation.
Salmonella spp.	Infrequent (1%)	Australian data 0.2 %, International data 0 %. Expert panel consultation.
S. aureus	Common (50%)	Australian data up to 23.3%, International data 0 – 96.2%. Expert panel consultation.

Table 7: Justification for contamination of raw sheep milk

Organism	Raw product contamination	Justification
C. jejuni	Rare (1:1000)	International data 0%. Expert panel consultation.
E. coli (EHEC)	Sometimes (10%)	International data 0 – 12.7%. Expert panel consultation.
L. monocytogenes	Sometimes (10%)	International data (Found in ewe's raw milk cheese 46%). Expert panel consultation.
Salmonella spp.	Rare (1:1000)	International data 0%. Expert panel consultation.
S. aureus	Sometimes (10%)	International data 7 – 33.3%. Expert panel consultation.

Appendix 2: Probabilistic growth models

This appendix summarises the growth equations used in the development of the predictive models for estimating the impact of cheese production and maturation on pathogen concentrations.

1 *Escherichia coli* growth model

A growth model equation for *E. coli* developed at the University of Tasmania was used in the process risk model (Ross *et al*., 2003). The general form of the model is given (Equation 1) and the model parameters in Table 1. This model includes terms for temperature (T) (°C), pH (pH), water activity (a_w), lactate concentration (LAC) (mM), where μ is the relative growth rate, the reciprocal of the generation time (h^{-1}).

$$\sqrt{\mu} = c(T - T_{\min}) \times (1 - \exp(d(T - T_{\min}))) \times \sqrt{a_w - a_{w\min}} \times \sqrt{1 - 10^{(\mathrm{pH}_{\min} - \mathrm{pH})}} \times \sqrt{1 - 10^{(\mathrm{pH} - \mathrm{pH}_{\max})}}$$
$$\times \sqrt{1 - [\mathrm{LAC}]/(U_{\min}(1 + 10^{(\mathrm{pH} - \mathrm{pK}_a)}))} \times \sqrt{1 - [\mathrm{LAC}]/(D_{\min}(1 + 10^{(\mathrm{pK}_a - \mathrm{pH})}))} + error$$

Equation 1

The model was developed using optical density data from three earlier studies: Salter (1998), Mellefont (2000) and Presser (2001). Parameter estimates are presented in Table 1.

Table 1: Growth model equation parameters for *E. coli* (Ross, 2003)

Parameter	Value	Parameter	Value
c	0.2345	$U_{\min}$	10.43
$T_{\min}$	4.14	$D_{\min}$	995.508
$T_{\max}$	49.55	$a_{w\,\min}$	0.9508
$\mathrm{pH}_{\min}$	3.909	d	0.2636
$\mathrm{pH}_{\max}$	8.86	pK_a	3.86

The reported root mean square error (RMSE) in $\sqrt{(1/(GT[h]))}$ for the model is 0.0054. The value from Ross (2003) is actually the square of the residual standard error; the correct RMSE is 0.2708.

2 *Staphylococcus aureus* growth model

A growth rate model for *S.s aureus* developed by Buchanan *et al.* (1993) was used in the risk model. A comparison between the growth rates estimates by this model and the *S. aureus* growth rate predictions in Pathogen Modelling Program (PMP, version 7) showed the model of Buchanan *et al.* (1993) is slightly more conservative, predicting a faster growth rate than PMP. The equation for generation time in general form is derived from the fitted parameters of a Gompertz function (Equation 2). A reduced version of the growth model is used in the risk assessment under the assumption that nitrite is neither used nor present during the manufacture of cheese. Equations 3 and 4 are the derived equations for parameters B and C based on the temperature (T, °C), pH (P), and salt concentration (S, % w/w).

$$\text{Generation Time (GT, min)} = \frac{Log_{10}2 \times e}{BC} \qquad \text{Equation 2}$$

$$\text{Ln (B)} = -10.8812 + 0.2551T + 1.0648P - 0.2653S - 0.00133TP + 0.00516TS - 0.00723PS - 0.00273T^2 - 0.0563P^2 + 0.00308S^2 \qquad \text{Equation 3}$$

$$Ln(MPN) = \ln(A + C) = 1.4074 + 0.00765T + 0.1588P + 0.0330S + 0.00241TP - 0.0000980TS - 0.00355PS - 0.000413T^2 - 0.0129P^2 - 0.00122S^2$$

Equation 4

The significant figures are as reported by Buchanan *et al.* (2003) .The correct equation for C, ignoring the contribution of sodium nitrite and assuming that A = 3, as used in the University of Tasmania report, is therefore:

$$C = \exp\begin{pmatrix} 1.4074 + 0.00765T + 0.1588P + 0.0330S + 0.00241TP - 0.0000980TS \\ - 0.00355PS - 0.000413T^2 - 0.0129P^2 - 0.00122S^2 \end{pmatrix} - 3$$

There are implications for the use of A = 3 in the application of the Gompertz equation that will not be considered in this report.

3 *Listeria monocytogenes* growth model

The growth rate for *L. monocytogenes* was modelled using an equation developed by Murphy *et al*. (1996) for growth in reconstituted skim milk. Growth media pH levels were adjusted by the addition of lactic acid. No information on the concentration of lactic acid was provided in the paper. The model was developed specifically for the prediction of growth of *L. monocytogenes* in dairy products. The growth model includes parameters for temperature (T), pH (P) and sodium chloride (S) concentration. Generation times are calculated with Equation 2, with parameters B and C calculated by Equation 5 and Equation 6, respectively.

$$\text{Ln (B)} = -48.0193 + 0.5612T + 0.1934S + 18.0587P - 0.0098T^2 - 0.0375S^2 - 2.6085P^2 - 0.0214TS - 0.0442TP + 0.1272P^3 + 0.0030TSP + 0.0008T^2P \qquad \text{Equation 5}$$

$$\text{Ln (C)} = -29.0536 + 0.0754T - 0.0674S + 13.4553P - 0.0025T^2 + 0.0165S^2 - 1.981P^2 - 0.0032TP + 0.00003T^3 - 0.0014S^3 + 0.0969P^3 \qquad \text{Equation 6}$$

An alternative growth rate model for *L. monocytogenes* that included lactate concentrations was also used in the development of the predictive model for Camembert cheese (Ross and Soontranon, 2006):

$$\sqrt{\mu} = c(T - T_{\min}) \times (1 - \exp(d(T - T_{\min}))) \times \sqrt{a_w - a_{w\min}} \times \sqrt{1 - 10^{(\mathrm{pH}_{\min} - \mathrm{pH})}} \times \sqrt{1 - [\mathrm{LAC}]/(U_{\min}(1 + 10^{(\mathrm{pH} - \mathrm{pK}_a)}))} + error$$

Equation 7

Parameter estimates for equation 7 are included in Table 2.

Table 2: Growth model equation parameters for *L. monocytogenes* (Ross and Soontranon, 2006)

Parameter	**Value**	**Parameter**	**Value**
c	0.15	$U_{\min}$	3.79
$T_{\min}$	0.88	$a_{w\,\min}$	0.0.923
$T_{\max}$	41.4	d	0.536
$\mathrm{pH}_{\min}$	4.97	pK_a	3.86

Appendix 3: Cheese classification schemes

Classification systems are primarily based on characteristics of the cheese including:

- Texture
- Method of coagulation
- Ripening indices

1 Textural classification schemes

Classification of cheese based essentially on moisture content has a long history. Schultz (1952) proposed a scheme which primarily consisted of five groups categorised by the moisture in fat-free cheese (MFFC) content (Schultz, 1952). The five groups were: dried (<40%), grated (40 - 49.9%), hard (50 - 59.9%), soft (60 - 69.9%) and fresh (70 - 82%). All but the dried group were then sub-divided into two sub-groups based on cooking and/or pressing. The sub-groups were later divided into six sub-sets (a - g) to reflect the rate and extent of acidification.

Davis (1965) suggested a number of possible classification schemes. One scheme was based primarily on rheological properties (moisture content), while a second scheme classified cheese into hard, semi-hard and soft (Davis, 1965) (Table 2). Varieties were then listed within each category according to milk type, method of coagulation, cutting of coagulum, scalding of the curds, drainage of whey and method of salting and moulding.

Walter and Hargrove (1972) suggested there were only 18 distinct types of natural cheese based on manufacturing technique, which they then grouped into eight families under the headings very hard, hard, semi-soft and soft (Walter and Hargrove, 1972) (Table 3).

(Scott, 1986) also classified cheeses primarily based on moisture content, *e.g.* hard, semi-hard and soft, and sub-divided these groups on the basis of cooking (scalding) temperature and/or secondary microflora (Table 4). Coagulation method was not considered with examples of rennet, acid and heat/acid coagulated cheeses included in some groups.

2 Coagulation based classification schemes

Generally there are three primary mechanisms for coagulating milk proteins to produce cheese: rennet, acid and heat/acid. (Fox, 2004) was the first to utilise coagulation method as a classification criterion when he suggested cheeses could be classified into super-families based on the coagulating agent:

- Rennet cheeses: Approximately 75% of total cheese produced and almost all ripened cheeses
- Acid cheeses: Approximately 25% of total cheese production generally consumed fresh *e.g.* Cottage, Quarg, Queso Blanco
- Heat/Acid cheeses: Limited varieties including Ricotta, Sapsago and Ziger

The original classification scheme of Fox (1993) was expanded and modified by Fox *et al*. (2000) which subdivided the rennet coagulated cheeses into further groups based on characteristic ripening agents or manufacturing technology. The most diverse family of rennet coagulated cheeses are the internal bacterially ripened varieties which are further

sub-divided based on moisture (extra hard, hard and semi-hard), the presence of eyes or a characteristic technology such as cooking/stretching or ripening under brine. Internal bacterially ripened cheese with eyes are further sub-divided into Swiss-type (lactate metabolism) or Dutch-type (citrate metabolism) types.

Fox *et* al. (2000) classifies natural cheese into internally bacterially ripened cheese (discussed above), mould ripened and surface ripened cheese categories. Mould ripened cheeses are then sub-divided into surface mould *e.g.* Brie and Camembert, and internal mould *e.g.* Roquefort, categories. Although fairly comprehensive, the classification scheme developed by Fox *et al.* (2000) does have limitations, some of these being: cheeses produced from the milk of different species, processed cheeses, cheese-based products and cheese analogues are all omitted.

3 Classification schemes based on ripening indices

During the 1960's, the possibility of classifying cheese using chemical fingerprints which develop during ripening was suggested (Davis, 1965). To date this is still not possible to achieve reliably and there is insufficient information, even on the major varieties of cheese, to permit classification using such complicated criteria.

4 Codex classification of cheese

Some cheeses are also classified by Codex on the basis of ripening and firmness. Ripening categories include: ripened, mould ripened, cheese in brine and unripened cheese. Firmness is based on percentage moisture on a fat free basis (MFFB %) and includes: soft (>67%), firm/semi-hard (54 - 69%), hard (49 - 56%) and extra hard (<51%).

Definitions are also provided for ripened, mould ripened and unripened cheese. Codex Standards applicable to cheese are outlined in Appendix 6.

5 Physicochemical characteristics of various cheese varieties

While the general principles of cheesemaking are common to most varieties of cheeses, no two batches of the same variety or probably no two cheeses are identical (Fox *et al*., 2004). Reported characteristics for selected cheeses however are outlined in the Table 1.

Table 1: Characteristics of various cheese varieties (Goff, 1995; Hill, 1995: Fox *et al*., 2000; Dairy Goodness, 2006)

Cheese	Moisture (%)	pH	Water activity
Cheddar	36-38.6	5.5	0.95
Colby	42	5.3	0.95 - 0.97
Brick	42	6.4	
Blue	47	6.5	0.96 - 0.99
Blue Stilton	38.6	5.2	0.96 - 0.99
Gorgonzola	41.3		0.95
Roquefort	41 - 47	6.4	0.91
Feta	55	4.4	0.96 - 0.99
Camembert	51 - 56	6.0-6.9	0.97

Table 2: Summary of fundamental cheese types (modified from Davis, [1965] scheme 2; Davis, 1965

Type	Cheese Variety	Characteristics	Milk		Method of Coagulation		Cutting			Scalding				Drainage		Salting			Shaping		
			Skimmed	Ripened	Acid	Rennet	Ladled	Large	Small	None	Low	Med	High	Vat	Hoop	Curd	Cheese	Brine	Hoop	Hand	Pressure
Very Hard	Parmesan	Very Hard	+	+		+			+				+				+	+			+
	Emmentaler	Large gas holes		+		+			+				+		+			+			+
	Cheddar	No gas holes		+		+			+			+		+		+					+
Semi-hard	Port du Salut	Fairly firm mild flavour	+	+		+			+			+						+			+
	Brick	Fairly strong sweetish flavour				+			+				+		+			+			+
	Pecorino	Sheep milk				+			+				+				+	+	+		
	Edam	Fairly firm	+			+		+			+				+		+	+			+
	Gouda	Mellow				+		+				+			+			+			+
	Caciocavallo	Full flavour, long keeping	+			+			+				+	+				+		+	
Soft surface smear	Cambridge	Unripened		+		+	+			+					+				+		
	Limburg	Strong flavour bacteria ripening	+			+			+	+					+			+	+		
Surface Mould	Camembert	Strong flavour, surface mould ripening				+	+								+			+	+		
Mould ripened (Blue veined)	Roquefort	Peppery flavour, internal mould ripening		+		+		+									+		+		
Acid Coagulated	Cottage	Soft lactic flavour	+	+	+			+				+		+		+			+		
	Sapsago	Flavoured by herbs	+	+	+								+			+			+		
Cream	Cream	Made from cream		+		+								+		+			+		

Table 3: Walter and Hargrove (1972) classification scheme (Walter and Hargrove, 1972)

1.	**Very hard (grating)**	
	1.1	Ripened by bacteria *e.g.* Asiago (old), Parmesan, Romano, Sapsago, Spalen
2.	**Hard**	
	2.1	Ripened by bacteria, without eyes *e.g.* Cheddar, Granular, Caciocavallo
	2.2	Ripened by bacteria, with eyes *e.g.* Emmentaler, Gruyère
3.	**Semi-soft**	
	3.1	Ripened principally by bacteria *e.g.* Brick, Münster
	3.2	Ripened by bacteria and surface micro-organisms *e.g.* Limburger, Port du Salut, Trappist
	3.3	Ripened principally by blue mould in the interior *e.g.* Roquefort, Gorgonzola, Danablu, Stilton, Blue Wensleydale
4.	**Soft**	
	4.1	Ripened *e.g.* Bel Paese, Brie, Camembert, Hand, Neufchatel
	4.2	Unripened *e.g.* Cottage, Pot, Baker's, Cream, Ricotta, Mysost, Primost

Table 4: Classification of cheese according to moisture content, cooking temperature and secondary microflora – adapted from Scott (1986)

Hard cheese (moisture content 20 - 42%)			
Low-scald **Ns**	**Medium-scald** **Ns**	**High-scald** **Ns or Pr**	**Plastic curds** **Ns or Pr**
Edam, Gouda, Cantal, Fontina, Cheshire	Cheddar, Glouchester, Derby, Leicester, Svecia, Dunlop, Turunmaa	Grana (Parmesan), Emmentaler, Gruyère, Beaufort, Herrgardsost, Asiago, Sbrinz	Scamorza, Provolone, Caciocavallo, Mozzarella, Kaaseri, Kashkaval, Perenica
Semi-hard cheese (moisture content 44-55%; low-scald)			
Ns	**Bs**	**Bv**	
St Paulin, Caerphilly, Lancashire, Trappist, Providence	Herve, Limburg, Romadur, Münster, Tilsit, Vacherin-Mont d'Or, Remoudou, Srainbuskerkase, Brick	Stilton, Roquefort, Gorgonzola, Danablu, Mycella, Wensleydale, Blue Vinny, Gammelost, Adelost, Tiroler – Graukäse, Edelpitzkäse, Aura, Cabrales	
Soft cheese (moisture content >55%; very low or no scald)			
Bs or Sm	**Sm**	**Ns**	**Un, Ac**
Bel Paese, Maroilles	Brie, Camembert, Carre d'est, Neufchatel, Chaource	Colwich, Lactic, Bondon	Coulommier, York, Cambridge, Cottage, Quarg Petit Suisse, Cream

Pr = propionic acid bacteria; **Ns =** normal lactic acid starter of milk flora; **Bs** = smear coat (*Brevibacterium linens* and other organisms); **Sm =** surface mould (*P. Camemberti*); **Bv** = Blue-veined interior mould (*P. roqueforti*); **Ac =** acid coagulated; **Un** = normally unripened, fresh cheese

Table 5: Classification of cheese according to Ottogalli (Ottogalli, 1998; Ottogalli, 2000a; Ottogalli, 2000b; Ottogalli, 2001)

Laciticinia Group			
Class	**Family**	**Description**	**Example**
Class A Fresh cheeses, rarely ripened	1	Yoghurt-like product, but with loss of some whey	Lebneh (Middle East)
	2	Milk coagulated by addition of organic acid	Queso Blanco (Latin America)
	3	Acid addition and heating of whey (goat or sheep)	Whey cheese (UK)
	4	Acid addition and heating of whey (cow)	Whey cheese (UK)
	5	Acid addition and heating of cream	Mascarpone (Italy)
	6	Acid addition and heating of buttermilk	Skyr (Iceland)
	7	Acid addition and heating of colostrum or beestings	Kolostrumkase (Germany)
Formatica Group			
Class	**Family**	**Description**	**Example**
Class B Fresh cheeses (unripened) Interior - soft, exterior – rindless Ripening – absent; IM = 2-5; IL = 1-2	1	Acid-rennet coagulation	Petit Suisse (France)
	2	Rennet-acid coagulation	Gervais™ (France)
	3	Goat or sheep	Caprino (Italy)
	4	Fresh-kneaded or plastic or stretched cheeses	Mozzarella di bufala (Italy)
	5	Coagulum cut into cubes and/or flakes cooked, drained, washed and water cooled.	Cottage (UK, USA)
Class C Short ripened cheeses Interior soft, exterior – usually rindless orthin rind; IM[1] = 2-10; IL[2] = 1-5	1	Rindless, very short ripening phase	Crescenza (Italy)
	2	Thin rind, short ripening (< 1 month)	Caciotta (Italy)
	3	Same as C1 or C2 but from goats or sheep milk	Burgos (Spain)
	4	Kneaded curds	Scamorza (Italy)
	5	White-brined	Feta (Greece)
Class D Soft surface ripened cheeses Interior – soft, exterior – felt of mould or smear; IM = 25-35; IL = 10-15	1	White-moulded rind	Camembert (France)
	2	Smear surface	Romadour (Belgium)
	3	Same as D1 or D2 or D4 but goats or sheep milk	Crottin (France)
	4	Mould-ripened (white or blue) and smeary surface	Taleggio (Italy)
Class E Blue-veinedCheeses Interior -soft to semi-soft, blue veins; exterior – soft rind with felt or smear; IM = 60-70; IL = 10-15	1	Cows milk	Buxton Blue (UK)
	2	White moulded rind	Bleu de Bresse (France)
	3	Sheep or goats milk	Roquefort (France)
Class F Semi-hard cheeses Interior –semi-hard, exterior – hard rind; IM = 10-15; IL = Depends on family	1	Untextured, usually semi-cooked and pressed	Montasio (Italy)
	2	Washed curd (eyes caused by citrate metabolism or heterolactic bacteria)	Edam (Netherlands)
	3	Same as F1 but from goats or sheep milk	Serra (PR)
	4	Kneaded curds (‘*pasta filata*’)	Caciocavallo (Italy)
	5	Propionic cheeses. Big round eyes	Maasdamer (Netherlands)
	6	Textured (and dry salted) curd	Lancashire (UK)
	7	Smeared rind	Fontina (Italy)
Class G Hard and extra-hard cheeses; Interior – hard, exterior- hard rind, long Ripened; IM = Depends on family; IL = Depends on family	1	Untextured, usually cooked and pressed	Asiago d’Allevo (Italy)
	2	Washed curd, long ripened	Gouda (Netherlands)
	3	Same as G1 but goats or sheep milk	Pecorino Romano (Italy)
	4	Kneaded curds (‘*pasta filata*’)	Provolone (Italy)
	5	Cheese with eyes	Emmentaler (Switzerland, France)
	6	Textured (and dry salted) curd (‘Cheddaring’)	Cantal (France)
	7	Smeared rind – the microbial coat causes the development of strong aroma	Tête de Moine (Switzerland)
Miscellanea Group			
Class	**Family**	**Description**	**Example**
Class H Cheeses made using various technologies	1	Melted	Processed cheese
	2	Smoked	Oak-smoked Cheddar (UK)
	3	Grated or fractionated	‘Grating cheeses’
	4	Mixed with other ingredients (fruit, vegetables, spices)	Friesan Clove cheese (NL)
	5	Ripened or kept under particular conditions. (*i.e.*, ‘Pickled cheeses’	Devon Garland (UK)
	6	Obtained using special technologies (*i.e.* ultrafiltration)	Philadelphia™ (USA)
	7	Products similar to cheese and with non-dairy ingredients	‘Imitation cheese’, filled cheese
Index of maturation (IM) = soluble Nitrogen x 100/total Nitrogen Index of lipolysis (IL) = free fatty acids x 100/total fat			

Table 6: Principal categories of cheese varieties (Fox *et al.*, 2000)

Internal Bacterially Ripened	
Extra-hard varieties *Italian "Grana" types, Asiago and "Pecorino" cheeses*	• Ripened for a long period (usually 6 - 24 months) • Hard granular texture and variable aromatic flavour • Use of semi-skimmed milk • High cooking temperature (*e.g.* Curds are scalded in vats for 20 - 30min at 50 - 55 °C) • Evaporation of moisture during ripening • These varieties can be consumed early during the ripening period as semi-hard cheeses
Hard varieties *Cheddar, British Territorial varieties include Cheshire, Derby, Gloucester and Leicester.*	• Moisture content range of 30 - 45% • High pressure during manufacture • Milk is coagulated using calf rennet or rennet substitute at about 30°C • Mesophilic starter culture to acidify milk – *Lactococcus* spp. • Coagulum is cut and cooked at 37 - 39 °C • Cheddaring the drained curd allows acidification to develop within. During this process the texture will become rubbery • For Cheddar, when the curd reaches a pH of 5.4, blocks are milled and dry salted • It may ripen in either an insulated room without temperature control or in a controlled environment at 4 - 8°C for 3months > 2years
Semi-Hard varieties *Colby, Monterey, Lancashire and Bryndza.*	• Stirring Cheddar-type cheese curd inhibits the development of curd structure and results in a cheese with higher moisture content and a softer texture
Cheese with eyes (Swiss type) *Maasdamer, Emmentaler and Jarlsberg*	• Main characteristic is eye formation – up to 2cm in diameter • Formation of eyes is achieved with the conversion of lactose to lactate to propionic acid, acetate and CO_2, with the aid of lactic acid bacteria and propionic acid bacteria • Biochemical reactions also produce the a nutty flavour within the cheese • Eye formation relies heavily on the following factors: o The curd must remain flexible and elastic in order to contain the gas resulting in eye formation o On ripening an optimum temp of 20 - 24°C must be held to allow propionic acid bacteria to rapidly grow and soften the cheese o Maintain low salt levels to aid in bacterial growth
Cheese with eyes (Dutch type) *Edam, Gouda*	• Eye formation occurs after citrate is catabolised to CO_2. Other bi-products include diacetyl and other volatile flavour compounds • Milk is acidified with the use of mesophilic, citrate-positive, starters. Coagulation is achieved with calf's rennet or equal substitute • When the coagulum has been cut and stirred, curds are effectively cooked when a portion of the whey is removed and replaced with hot water • After whey drainage and cooking at 36 - 38°C, curds are pressed and brine salted. They are they coated with wax and matured for 2 - 3 months (or longer) at 15°C.
Pasta-filata cheeses *(kneaded or plastic curd) Mozzarella, Provolone and Kasseri*	• Milk is coagulated with calf rennet and acidified with *Streptococcus thermophilus* • *Lactobacillus* spp. are utilised as a starter culture • Coagulum is cut and cooked at 41°C, then drained, allowed to acidify then heated, kneaded and stretched
Cheeses ripened under brine *Feta, Domiati*	• Coagulation is achieved using rennet and acidification is achieved using either thermophilic or mesophilic lactic bacteria as a started culture • Coagulum is cut without cooking and left to drain until cohesion occurs • Cheese is cut into pieces and salted and transferred to brine solution to allow ripening. This is carried out at 14 - 16°C for 7 days and the pH has dropped to 4.5 • Cheese is then stored at 3 - 4°C for at least 2 months

Table 6 cont: Principal categories of cheese varieties (Fox *et al.*, 2000)

Mould ripened varieties	
Surface mould ripened varieties	These soft cheeses are characterised by the growth of *Penicillium Camemberti* on the cheese surface. • Mesophilic starter culture acidifies curd to around pH 4.6 • Mould spores may be added to the milk or sprayed onto the cheese post-production • Coagulation is achieved using rennet • On coagulation, the product is ladled directly into mould to aid draining • Brine salted and ripened for 10-12 days at 12°C to enable mould formation • Surface pH is around 7.0 due to metabolism of lactic acid by mould • Ripening occurs with the action of lactate catabolism at the surface of the cheese aided by mould, facilitating the migration of lactate from the core • Softening occurs with migration of soluble calcium phosphate from the core of the cheese to the outer surface. Proteolysis plays a minor role in softening
Internal mould ripened varieties *Cabrales, Gorgonzola and Stilton as well as Roquefort*	• Coagulation achieved with rennet extract • Curd acidification by mesophilic lactic culture. Curds cooked at low temperatures before transferring to moulds • Either dry-salted or brine-salted. • Mechanical openings in cheese (not pressing or piercing) to supply oxygen for *Penicillium roqueforti* growth • Ripened in aerobic conditions to favour mould growth (the interior of the cheese is made aerobic with the puncturing of the surface with needles) • Ripening is characterised by extensive lipolysis. And flavour is attributed to n-methyl ketones produced via fatty acids from the mould. • pH increases during ripening from 4.6 - 5.0 to 6.0 - 6.5
Surface smear ripened varieties	
	These cheeses are also referred to as smear cheeses and are characterised by the growth of complex Gram-positive microflora on the surface during ripening. Although most varieties in this group are soft or semi-hard, a surface flora may also develop on hard cheeses such as Gruyère. • Development of mixed microflora on cheese surface forming a red-orange smear • Characterised by strong aromas and high levels of both proteolysis and lipolysis mainly on the surface • Generally brine salted, cooked at a low temperature and acidified using mesophilic (most varieties) or thermophilic (Gruyère) culture • Moulded into cylindrical shapes, increasing the surface area to allow the 'smear' to have a large effect on mature cheeses. High moisture content also affects the characteristics of mature cheeses • During manufacture the cheese is washed periodically in brine solution, often in a practise referred to as 'old-young smearing'. In this case the brine used to wash the more mature cheeses are then later re-used to wash the younger cheeses to create an intense build up of microflora • Yeast dominates the micro flora following manufacture and act to de-acidify the cheese surface and encourage the growth of Coryneform bacteria
Acid-curd cheese	
	Acid-coagulated cheese is the variety in which milk is acidified to a pH of 4.6 resulting in coagulation. • Acidification achieved with mesophilic starter cultures. Direct acidification may also be practised • High water content • Consumed fresh however they may be ripened • Dehydration of curds (removal of whey) during production • Small amounts of rennet may be included (increase firmness of the coagulant and minimise casein loss in the whey) *e.g.* Cottage or Quarg • Coagulum is not pressed, but may or may not be cut
Heat/Acid cheese	
Ricotta	• Milk is acidified to about pH 6.0 • Acidified milk heated to around 85 - 90^{o}C • Separation of curds and whey

Appendix 4: Foodborne illness associated with consumption of raw milk cheeses

Table 1: Outbreaks of illness associated with raw cow milk cheese

Year	Country	Cases (death)	Product	Causative Agent	Comment	Reference
2005	Switzerland	10 (5)	Tomme Soft Cheese	*Listeria monocytogenes*	-	Bille *et al.* 2006
2001 - 2004	USA	35 (1)	Fresh cheese (queso fresco)	*Mycobacterium bovis*	Illegally imported raw milk cheese from Mexico	(CDC, 2005)
2003	Sweden	15	Fresh cheese	*Listeria monocytogenes*	On farm manufactured fresh cheese	(Carrique-Mas *et al*., 2003)
2002	Canada	17	Raw milk cheese	*Listeria monocytogenes*	Environmental contamination	(CCDR, 2003)
2002	Canada	13	Unpasteurised gouda cheese	*E. coli* O157:H7	Implicated cheese was found to be contaminated with *E. coli* O157:H7 104 days after production, despite having met regulated microbiological and aging requirements	(Honish *et al*., 2005)
2001 (Oct)	France	25	Cantel cheese	*Salmonella* Enteritidis phage type 8	Cheese made from raw milk - cross contamination from cellar due to previous outbreak suggested as possible cause	(Haeghebaert *et al*., 2003)
2001 (June-July)	France	190	Cantel cheese	*Salmonella* Enteritidis phage type 8	Cheese made from raw milk	(Haeghebaert *et al*., 2003)
2000	USA	12 (5 still births)	Mexican style cheese	*Listeria monocytogenes*	Mexican-style cheese made from contaminated raw milk traced to 1 local dairy	(CDC, 2003; MacDonald *et al*., 2005)
1999	Brazil	50	Raw milk homemade white Minas cheese	*Staphylococcus aureus*	High level of *S. aureus* toxins were produced	(do Carmo *et al*., 2002)
1998	Canada	17	Raw milk cheese	*Listeria monocytogenes*	-	(CCDR, 2003)
1998	USA	55	Fresh cheese curds	*E. coli* O157:H7	Produced during manufacture of Cheddar cheese from unpasteurised milk and had been incorrectly labelled as pasteurised	(Durch *et al*., 2000)
1997	UK	2	Lancashire-type cheese	*E. coli* O157:H7	-	(Anon, 1997a)
1997	USA	54	Mexican style soft cheese made with unpasteurised milk	*Salmonella* Typhimurium DT104	Raw milk samples from nearby dairies yielded *Salmonella* Typhimurium DT104	(Villar *et al*., 1999)
1997	USA	31	Unpasteurised Mexican style soft cheese	*Salmonella* Typhimurium var Copenhagen DT104	Fresh Mexican-style cheese from street vendors and from cheese samples and raw milk	(Cody *et al*., 1999)
1997	USA	79	Mexican style soft cheese made with unpasteurised milk	*Salmonella* Typhimurium DT104	Consumption of fresh Mexican cheese made from raw milk	(Cody *et al*., 1999)
1997	France	14	Livarot, Pont-L'eveque cheese (soft cheese)	*Listeria monocytogenes*	Raw milk cheese	(Jacquet *et al*., 1998)

Table 1 cont: Outbreaks of illness associated with raw cow milk cheese

Year	Country	Cases (death)	Product	*Causative Agent*	Comment	Reference
1997	France	113	Raw milk soft cheese	*Salmonella enterica* Typhimurium	From a single processing plant	(De Valk *et al*., 2000)
1996	Spain	81	Raw cheese	*Brucella melitensis*	Use of raw cows milk in the product (home-made)	(Castell *et al*., 1996)
1996	France	14 (1)	Mont d'Or cheese	*Salmonella* Dublin	Cheese made from raw cows milk	(Infuso *et al*., 1997)
1995	Malta	135 (1)	Raw milk soft cheese	*Brucella melitensis*	Associated with unpasteurised milk	(Anon, 1995)
1995	France	36 (4)	Brie de Meaux cheese	*Listeria monocytogenes*	-	(Vaillant *et al*., 1998) from (De Buyser *et al*., 2001)
1995	France	25 (5)	Mont d'Or cheese (soft cheese)	*Salmonella* Dublin	Cheese made from raw cows milk	(Vaillant *et al*., 1996)
1995	Germany	14	Raw milk cheese	*Brucella* spp.	Consumption of raw milk cheese	(Rasch *et al*., 1997)
1994	Canada	82	Unpasteurised soft cheese	*Salmonella* Berta	Contaminated of cheese by chicken carcasses during production	(Ellis *et al*., 1998)
1994	Brazil	7	Raw milk cheese	*Staphylococcus aureus*	Enterotoxin H found	(Pereira *et al*., 1996)
1994	Scotland	22	Raw milk cheese	*E. coli* O157	Local farm produced cheese - possibly raw	(Ammon, 1997)
1989	England	42	Irish soft cheese	*Salmonella* Dublin	Unpasteurised milk	(Maguire *et al*., 1992)
1988	UK	155	Stilton Cheese	Suggestive of a staphylococcal illness	Stilton cheese, produced from unpasteurised cow's milk	(Maguire *et al*., 1991)
1985	Finland	35	Raw farm cheese	*Salmonella* spp.	-	(Huchot *et al*., 1993) from (De Buyser *et al*., 2001)
1985	USA	9	Mexican style cheese (Quesco fresco)	*Brucella melitensis*	Illegally imported, raw milk suspected	(Boor and Zadoks, 2003) (Altekruse *et al*., 1998)
1985	France	>40	Vacherin Mont d'Or cheese	*Salmonella* typhimurium	-	(Sadik *et al*., 1986)
1984-1985	Switzerland	215	Vacherin Mont d'Or cheese	*Salmonella* typhimurium	Hand based contamination from a pigsty	(Sadik *et al*., 1986)
1983 - 1987	Switzerland	122 (34)	Vacherin Mont d'Or cheese	*Listeria monocytogenes*	A cheese made from thermised milk	(Bille, 1990); (Bula *et al*., 1995)
1983	USA	16	Mexican style soft cheese (queso fresco)	*Streptococcus equi* and *Streptococcus zooepidericus*	Raw milk used in production of cheese	(Altekruse *et al*., 1998)
1983	USA	45	Brie cheese	Enterotoxigenic *E. coli*	Consumption of imported raw milk French Brie cheese at an office party	(MacDonald *et al*., 1985)
1982	Canada	-	Cheddar cheese	*Salmonella muenster*	Unpasteurised cheese	(D'Aoust, 1985)
1975	USA	17	Mexican style soft cheese – (queso fresco)	Unknown organisms	Illegally imported raw milk cheese	(Altekruse *et al*., 1998)
1973	USA	3	Mexican style soft cheese (queso fresco)	*Brucella melitensis*	Illegally imported, raw milk suspected	(Altekruse *et al*., 1998)

Table 2: Outbreaks of illness associated with raw goat milk cheese

Year	Country	Cases (death)	Product	Causative Agent	Comment	Reference
2006	Finland	7	Soft unpasteurised goats milk cheese	*Streptococcus equi* subspecies *zooepidemicus*	Small farm production	(Kuusi *et al*., 2006)
2006	France	3	Raw goats milk cheese	*E. coli* O157	Unpasteurised raw goat cheese produced by a local provider	(Espie *et al*., 2006)
2005	France	18	Raw goats milk cheese	*Salmonella* Stourbridge	Cheese was made from the unpasteurised milk of a single herd of 260 goats	(Vaillant *et al*., 2005)
2004	Italy	4	Unpasteurised goats milk cheese	*Brucella melitensis*	Symptoms included fever and lumbar pain	(Taliani *et al*., 2004)
2002	Spain	11	Raw goats cheese	*Brucella melitensis* serovar 3	Unpasteurised raw goat cheese produced in a farmhouse	(Mendez *et al*., 2003)
1999	Canada	?	Cheese from goats milk	*Coxiella burnetii*	Associated with contact with goat placenta, smoking tobacco	(Hatchette *et al*., 2001)
1995	Malta	135 (1)	Soft cheese made with raw goats milk	*Brucella melitensis*	Consumption of raw milk cheese	(Anon, 1995)
1994	France	4	Raw goats milk cheese	*E. coli* O103	Goats milk suspected	(Ammon, 1997)
1993	France	273 (1)	Unpasteurised goats milk cheese	*Salmonella enterica* Paratyphi B phage type 1 var 3	Brand A unpasteurised goats' milk cheese	(Desenclos *et al*., 1996)
1992	France	4 (1)	Unpasteurised fromage frais (mixed goat and cow milk cheese	Verotoxin 2 gene detected by PCR (suggestive of *E. coli* intoxication)	Acute haemolytic uraemic syndrome (HUS)	(Deschenes *et al*., 1996)
1992	France	40	Raw goats milk	*Coxiella burnetii*	Persons who worked on farm and consumed unpasteurised milk products	(Fishbein and Raoult, 1992)
1990	France	277	Contaminated goats milk cheese	*Salmonella enterica* Paratyphi B	Out break was possibly related to contaminated goats milk cheese	(Desenclos *et al*., 1996) from (Grimont and Bouvet, 1991))
1988	England	1	Goats milk soft cheese	*Listeria* spp.	Immunocompromised case	(Azadian *et al*., 1989)
1983	USA	31	Raw goats cheese	*Brucella melitensis*	Mexican raw goats milk cheese	(Thapar and Young, 1986)
1973	USA	3	Mexican fresh raw cheese	*Brucella melitensis*	Mexican raw goats milk cheese	(Eckman, 1975)
1973	Mexico	6	Fresh raw goats cheese	*Brucella melitensis*	Mexican raw goats milk cheese	(Young and Suvannoparrat , 1975)

Table 3: Outbreaks of illness associated with raw sheep milk cheese

Year	Country	Cases	Product	Causative Agent	Comment	Reference
1988	Czech Republic	74	Non pasteurised sheep milk cheese	*Campylobacter jejuni/coli*	Cheese prepared from unpasteurised sheep milk	(Kourilova and Kultan, 1990)
1984-1985	Scotland	27	Sheep raw milk cheese	*Staphylococcus aureus*	Contamination of cheese with detectable enterotoxins present, no viable organisms	(Bone *et al*., 1989)
1983	France	20	Sheep milk cheese	*Staphylococcus aureus*	Made with raw sheep milk, shepherd asymptomatic carrier of *S. aureus*	(De Buyser *et al*., 1985)
1980	Czechoslovakia	Several hundred	Sheep milk cheese	*Shigella* spp.	Caused by a dairy worker	(Nunez *et al*., 1989))

Table 4: Outbreaks of illness associated with pasteurised milk cheeses

Year	Country	Cases (death)	Product	Causative Agent	Comment	Reference
2001	France	45	Brie cheese	Salmonellosis serotype infantis	Milk and factory workers contaminated with *Salmonella* serotype infantis	(Simon *et al*., 2002)
1998	Canada	~700	Cheddar cheese	*Salmonella enteritidis*	Pasteurised Cheddar cheese - contamination	(CCDR, 1999)
1996	Italy	8	Mascarpone cheese	*Clostridium botulinum* type A	Break in cold-chain at retail likely caused germination of *C. botulinum* spores contaminating the products	(Aureli *et al*., 2000)
1996	UK	84	Cheddar cheese	*Salmonella* Goldcoast	Failure in pasteurisation	(Anon, 1997b)
1995	Switzerland	57 (16)	Soft cheese	*Listeria* spp.	Consumption of a soft cheese	(Bula *et al*., 1995)
1995	USA	9	Cheese	*Clostridium perfringens*	Consumed in ra estaurant	(CDC, 2002)
1994	USA	5	Goats cheese	*Salmonella* enteritis	Consumed in a private home	(CDC 2002)
1993	USA	12	Cheese slices	Unknown	Consumed at a picnic	(CDC 2002)
1991	USA	25	Shredded cheese	Unknown	Consumed in a restaurant	(CDC 2002)
1990	USA	23	Cheese sauce	*Salmonella* Braenderup	Consumed in a restaurant	(CDC 2002)
1990	USA	12	Processed Cheese	*Salmonella* Enteritidis	Consumed in a hospital	(CDC 2002)
1989	USA	164	Mozzarella	*Salmonella* javiana and Salmonella Oranienberg	Contaminated cheese - poor sanitation in cheese processing plant, post pasteurisation	(Altekruse *et al*., 1998; Hedberg *et al*., 1992)
1986	USA	339	Uncured Cheddar cheese	*Salmonella* Heidelberg	Improper pasteurisation	(Altekruse *et al*., 1998)
1985	USA	152 (48-52)	Mexican style cheese (Quesco fresco)	*Listeria monocytogenes*	Improper pasteurisation	(Altekruse *et al*., 1998; Linnan *et al*., 1988)

Table 4 cont: Outbreaks of illness associated with pasteurised milk cheeses

Year	Country	Cases (death)	Product	Causative Agent	Comment	Reference
1984	Canada	>2700	Cheddar cheese	*Salmonella* typhimurium PT10	Pasteuriser manually shut down by staff - raw milk used in cheesemaking process	(Bezanson *et al*., 1985; D'Aoust, 1985)
1983	UK	2	Cheese	*Staphylococcus aureus*	Pasteurised product	(Barrett, 1986)
1981	USA	16	Hand pressed set cheese	*Staphylococcus aureus*	Pasteurisation failed	(Altekruse *et al*., 1998)
1981	USA	321 (2)	Mozzarella	*Salmonella* typhimurium	Pasteurisation failed	(Altekruse *et al*., 1998)
1976	USA	28,000 - 36,000	Cheddar cheese	*Salmonella* Heidelberg	Consumption of Cheddar cheese from a single shipment of a single manufacturer, deficiencies in pasteurisation procedures	(Fontaine *et al*., 1980)
1995 - 1996	Spain	>200	Fresh Pasteurised milk cheese	*Shigella sonnei*	Likely source of contamination - food handler cross contamination	(Garcia-Fulgueiras *et al*., 2001)

Table 5: Cheeses unspecified or unknown, manufactured from raw or pasteurised milk

Year	Country	Cases (death)	Product	Causative Agent	Comment	Reference
2001	France	45	Brie cheese	*Salmonella* Infantis	Brie made with contaminated milk	(Simon *et al*., 2002)
1989-1990	Denmark	26 (6)	Blue mould hard cheese	*Listeria monocytogenes*	Unknown	(Jensen *et al*., 1994; Ryser and Marth, 1999)
1989	Luxemburg	2	Camembert	*Listeria monocytogenes*	Unspecified	(Ries *et al*., 1990)
1983	USA	170	Brie/Camembert cheese	*E. coli* O27:H20	Also caused illness in Denmark, Netherlands (69) and Sweden (66), was a pasteurised product	(Altekruse *et al*., 1998) (De Buyser *et al*., 2001; MacDonald *et al*., 1985)
1981	Italy	>100	Mozzarella	*Salmonella* spp.	Unspecified	(Huchot *et al*., 1993)
1980	Canada	62	Cheese curd	*Staphylococcus aureus*	Unspecified	(Todd *et al*., 1981)

APPENDIX 5: Prevalence/incidence of microbiological hazards in raw milk cheeses

1 Prevalence of pathogens in raw milk cheese

1.1 Raw cow milk cheese

Table 1: Prevalence of *Brucella* spp.in raw cow milk cheese

Organism Isolated	Cheese type	Country	Samples	% Positive	Reference
Brucella spp.	Cheese (unspecified)	Turkey	35	0	(Kasimoglu, 2002)

Table 2: Prevalence of *Campylobacter* spp. in raw cow milk cheese

Organism Isolated	Cheese type	Country	Samples	% Positive	Reference
Campylobacter spp.	Milk and other dairy products	Switzerland	93	6.5 (PCR) 0(culture)	(Wegmuller *et al*., 1993)
Campylobacter spp.	Mexican Mennonite style cheese	USA	8	0	(Bricker *et al*., 2005)
Campylobacter spp.	Raw milk cheese – retail sample	Europe	509	0	(Anon, 2004a)
Campylobacter spp.	Raw milk cheese – processing sample	Europe	28	0	(Anon, 2004a)

Table 3: Prevalence of *E. coli* in raw cow milk cheese

Organism Isolated	Cheese type	Country	Samples	% Positive	Reference
E. coli O157	Dairy products	Italy	811	0.1	(Conedera *et al*., 2004)
E. coli O157	Cheese (unspecified)	Scotland	739	0	(Coia, 2001)
E. coli O157	White pickled cheese	Turkey	50	4	(Oksuz *et al*., 2004)
E. coli O157	Soft and semi soft cheeses	USA	19	0	(Ansay and Kaspar, 1997)
E. coli O157	Fungal ripened soft cheese	Belgium	71	5.6	(De Reu *et al*., 2002)
E. coli O157	Raw milk cheese products	Belgium	16	0	(De Reu *et al*., 2002)
E. coli O157	Mexican Mennonite style cheese	USA	8	0	(Bricker *et al*., 2005)
E. coli O157	Turkish Van otlu cheese (Unripened)	Turkey	50	0	(Kaan Tekinsen and Ozdemir, 2006)
E. coli O157	Direct marketing German soft and semi-hard cheese	Germany	334	0	(Hahn *et al*., 1999)
E. coli O157	Cheeses made from raw/ thermised milk	UK	801	0	http://www.food.gov.uk/multimedia/pdfs/cheeses.pdf

Table 3 cont.: Prevalence of *E. coli* in raw cow milk cheese

Organism Isolated	Cheese type	Country	Samples	% Positive	Reference
E. coli VTEC	Direct marketing German soft and semi hard cheese	Germany	334	1.5	(Hahn *et al*., 1999)
E. coli - Pathogenic strains	Surface mould ripened soft cheese - Brie	Netherlands	92	0	(Nooitgedagt and Hartog, 1988)
E. coli - Pathogenic strains	Surface mould ripened soft cheese - Camembert	Netherlands	89	0	(Nooitgedagt and Hartog, 1988)
E. coli	Raw milk cheese – retail sample	Europe	509	0.6	(Anon, 2004a)
E. coli	Raw milk cheese – processing sample	Europe	28	0.00	(Anon, 2004a)
E. coli	Turkish unripened Van otlu cheese	Turkey	50	62	(Kaan Tekinsen and Ozdemir, 2006)
E. coli	Soft and semi soft cheeses	USA	19	58	(Ansay and Kaspar, 1997)
E. coli	Soft cheeses	Belgium	47	81	(De Reu *et al*., 2002)
E. coli	Tetilla – (Spanish) cheese	Spain	24	25	(Menendez *et al*., 2001)
E. coli	Surface mould ripened soft cheese - Brie	Netherlands	181	4.9	(Nooitgedagt and Hartog, 1988)
STEC	Various French raw milk cheeses	France	1039	13.1	(Vernozy-Rozand *et al*., 2005)
STEC	French soft cheeses	France	1039	7.1	(Vernozy-Rozand *et al*., 2005)
STEC	French uncooked hard cheese	France	1039	5.2	(Vernozy-Rozand *et al*., 2005)
STEC	French raw milk cheese	France	180	30.5	(Fach *et al*., 2001)
STEC	French raw milk cheese	France	603	9.9	(Pradel *et al*., 2000)
Toxigenic *E. coli*	Cheese – raw milk, soft	Spain	221	1.4	(Quinto and Cepeda, 1997)

Table 4: Prevalence of *Listeria* spp. in raw cow milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
L. monocytogenes	Raw milk cheeses	Belgium	71	2.8	(De Reu *et al*., 2002)
Listeria spp.	Raw milk cheeses	Belgium	71	5.6	(De Reu *et al*., 2002)
L. monocytogenes	Semi-hard - hard cheese	Belgium	16	0	(De Reu *et al*., 2002)
Listeria spp.	Semi-hard - hard cheese	Belgium	16	6.2	(De Reu *et al*., 2002)
L. monocytogenes	Raw milk craft cheese – (114 artisan, 39 industrial)	Belgium	153	7.2	(Vivegnis *et al*., 1998)
L. monocytogenes	Canadian soft cheeses	Canada	19	0	(Farber *et al*., 1987)

Table 4 cont.: Prevalence of *Listeria* spp. in raw cow milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
L. monocytogenes	French cheeses - pasteurisation unknown	Canada	104	1.9	(Farber *et al*., 1987)
L. monocytogenes	Mexican Mennonite style cheese	USA	8	0	(Bricker *et al*., 2005)
L. monocytogenes	Tulum cheese (semi-hard)	Turkey	250	4.8	(Colak *et al*., 2006)
L. monocytogenes	Tetilla – (Spanish) cheese	Spain	24	8.3	(Menendez *et al*., 2001)
L. monocytogenes	Raw or mild heat treated milk cheeses	Ireland	75	0	(Coveney *et al*., 1994)
L. monocytogenes	Direct marketing German soft and semi hard cheese	Germany	334	2.4	(Hahn *et al*., 1999)
L. monocytogenes	Red smear cheese	Germany	166	4.8	(Rudolf, 2001)
L. monocytogenes	Raw milk cheese – retail sample	Europe	507	2.9	(Anon, 2004a)
L. monocytogenes	Raw milk cheese – processing sample	Europe	28	0	(Anon, 2004a)
L. monocytogenes	Cheese (unspecified)	Sweden	31	41.9	(Loncarevic *et al*., 1995)
L. monocytogenes	Cheese (unspecified)	Netherlands	14	64.3	(Beckers *et al*., 1987)
Listeria spp.	Soft cheese	UK	222	10	(Zottola and Smith, 1991)
Listeria spp.	Gorgonzola	UK	864	8.45	(Zottola and Smith, 1991)
Listeria spp.	Taleggio	UK	864	13.7	(Zottola and Smith, 1991)
L. monocytogenes	Soft cheese	France	-	65	(Beckers *et al*., 1987)
L. monocytogenes	Soft ripened cheese	England and Wales	-	8.2	(Greenwood *et al*., 1991)
L. monocytogenes	Soft unripened cheese	England and Wales	-	1.1	(Greenwood *et al*., 1991)
L. monocytogenes	Soft cheese	Italy, Germany, Austria and France	-	6	(Rudolf, 2001)
L. monocytogenes	Soft or semi-soft cheese	France, Germany and Italy	-	6	(Loncarevic *et al*., 1995)
L. monocytogenes	Semi-soft cheese	Italy, Germany, Austria and France	-	8	(Rudolf, 2001)
L. monocytogenes	Hard cheese	England and Wales	-	1.5	(Greenwood *et al*., 1991)
L. monocytogenes	Hard cheese	Italy, Germany, Austria and France	-	4	(Rudolf, 2001)

Table 5: Prevalence of *Salmonella* spp. in raw cow milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
Salmonella spp.	Raw milk cheeses	Belgium	71	0	(De Reu *et al*., 2002)
Salmonella spp.	Mexican Mennonite style cheese	USA	8	0	(Bricker *et al*., 2005)
Salmonella spp.	Raw milk craft cheese	Belgium	153	0.7	(Vivegnis *et al*., 1998)
Salmonella spp.	Raw milk tulum cheese (semi hard)	Turkey	250	2.4	(Colak *et al*., 2006)
Salmonella spp.	Carra (Turkish) cheese	Turkey	50	0	(Aygun *et al*., 2005)
Salmonella spp.	Tetilla – (Spanish) cheese	Spain	24	0	(Menendez *et al*., 2001)
Salmonella spp.	Surface mould ripened soft cheese - Brie and Camembert	Netherlands	181	0	(Nooitgedagt and Hartog, 1988)
Salmonella spp.	Semi-hard - hard cheese	Belgium	16	0	(De Rue *et al*., 2004)
Salmonella spp.	Raw or mild heat treated milk cheeses	Ireland	75	0	(Coveney *et al*., 1994)
Salmonella spp.	Raw milk cheese (79% farm produced, 21% factory produced)	France	2350	0.2 (all farm produced)	(De Buyser *et al*., 2001)
Salmonella spp.	Direct marketing German soft and semi hard cheese	Germany	334	0	(Hahn *et al*., 1999)
Salmonella spp.	Raw milk cheese – retail sample	Europe	506	0	(Anon, 2004a)
Salmonella spp.	Raw milk cheese – processing sample	Europe	28	0	(Anon, 2004a)
Salmonella spp.	Turkish unripened Van otlu cheese	Turkey	50	6.0	(Kaan Tekinsen and Ozdemir, 2006)

Table 6: Prevalence of *Staphylococcus* spp. in raw cow milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
Staphylococcal enterotoxin	Mexican Mennonite style cheese	USA	8	0	(Bricker *et al*., 2005)
S. aureus	Unripened cheese produced from raw milk	Sweden	37	30	(Sylven, 1998) from (Lindqvist *et al*., 2002)
S. aureus enterotoxins	Raw milk cheeses	Belgium	71	1.4	(De Reu *et al*., 2002)
S. aureus	Ricotta, raw cow's milk	Italy	32	0	(Cosseddu *et al*., 1997)
S. aureus	Tetilla – (Spanish) cheese	Spain	24	12.5	(Menendez *et al*., 2001)
S. aureus	Turkish unripened Van otlu cheese	Turkey	50	100	(Kaan Tekinsen and Ozdemir, 2006)
S. aureus	Surface mould ripened soft cheese - Brie and Camembert	Netherlands	181	2.2	(Nooitgedagt and Hartog, 1988)
S. aureus	Semi-hard - hard cheese	Belgium	16	18.7	(De Rue *et al*., 2004)
S. aureus	Raw milk cheese – retail sample	Europe	511	5.4	(Anon, 2004a)
S. aureus	Raw milk cheese – processing sample	Europe	28	28.5	(Anon, 2004a)

1.2 Raw goat milk cheese

Table 7: Prevalence of *Brucella* spp. in raw goat milk cheese

Organism Isolated	Cheese type	Country	Samples	% Positive	Reference
Brucella spp.	Cheese (unspecified goat and sheep milk)	Italy	46	46	(Tantillo *et al*., 2001)

Table 8: Prevalence of *Salmonella* spp. in raw goat milk cheese

Organism Isolated	Cheese type	Country	Samples	% Positive	Reference
Salmonella spp.	Raw milk goat cheese	Spain	24	0	(Mor-Mur *et al*., 1992)

1.3 Raw sheep milk cheese

Table 9: Prevalence of *Brucella* spp. in raw sheep milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
Brucella spp.	Cheese (unspecified)	Turkey	35	14.2	(Kasimoglu, 2002)
Brucella spp.	Cheese – sheep and goat milk	Italy	46	46	(Tantillo *et al*., 2001)

Table 10: Prevalence of *E. coli* in raw sheep milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
E. coli O157	Raw sheep products	Italy	502	0	(Conedera *et al*., 2004)
E. coli O157	Raw sheep milk	Spain	84	3.6	(Caro *et al*., 2006)

Table 11: Prevalence of *Listeria* spp, in raw sheep milk cheese

Organisms Isolated	Sample type	Country	Samples	% Positive	Reference
L. monocytogenes	Soft cheese	Portugal	63	46	(Pintado *et al*., 2005)
L. innocua	Soft cheese	Portugal	63	29	(Pintado *et al*., 2005)
Listeria spp.	Soft cheese	Portugal	63	75	(Pintado *et al*., 2005)

2 Incidence of pathogens in raw milk cheese

Internationally, many different pathogenic organisms have been identified in studies examining raw milk cheeses. It is difficult to compare the results of individual studies due to differences in countries of origin, types of cheese and processing techniques, sampling during various stages of production, types of organisms tested for and different enumeration methodologies.

The different physical, chemical, microbiological and processing conditions between different cheese types and indeed within cheese types make it difficult to compare the level and survival of pathogen contamination of the cheeses. Pathogens present in raw milk used for different types of cheese production may produce vastly different microbial levels after the cheesemaking process is complete.

2.1 Raw cow milk cheese

Table 12: Coliforms in raw milk cheeses

Cheese Type	No. Samples	Min	Mean	Median	Max	Reference
Mould ripened soft cheese	34	<10	-	-	1.0×10^7	(De Reu *et al*., 2002)
Red smear soft cheese	14	30	-	-	$>3 \times 10^6$	(De Reu *et al*., 2002)
Blue veined cheese	8	<10	-	-	2.0×10^3	(De Reu *et al*., 2002)
Semi-hard cheese	2	<10	-	-	90	(De Reu *et al*., 2002)
Hard cheese	4	<10	-	-	2.5×10^3	(De Reu *et al*., 2002)
Fresh cheese	8	<10	-	-	270	(De Reu *et al*., 2002)
Unspecified raw milk cheese	50	<1	-	3.0×10^4	10^5	(Oksuz *et al*., 2004)
Fresh Turkish cheese	100	7.5×10^3	-	-	1.1×10^5	(Yucel and Ulusoy, 2006)
Carra cheese	50	<100	1.02×10^4	<100	1.9×10^5	(Aygun *et al*., 2005)

Table 13: *E. coli* in raw milk cheeses

Cheese Type	No. Samples	Min	Mean	Median	Max	Reference
Mould Ripened soft cheese	34	<10	-	-	1.1×10^6	(De Reu *et al*., 2002)
Red smear soft cheese	14	10	-	-	3.2×10^6	(De Reu *et al*., 2002)
Blue veined cheese	8	<10	-	-	40	(De Reu *et al*., 2002)
Semi-hard cheese	2	<10	-	-	200	(De Reu *et al*., 2002)
Hard cheese	4	<10	-	-	3.3×10^3	(De Reu *et al*., 2002)
Fresh cheese	8	<10	-	-	240	(De Reu *et al*., 2002)
Fresh Turkish cheese	100	3.6×10^2	-	-	1.1×10^5	(Yucel and Ulusoy, 2006)
Tetilla cheese	24	-	-	-	5.25×10^1	(Menendez *et al*., 2001)
Turkish Van otlu cheese	50	0	4.79×10^3	-	-	(Kaan Tekinsen and Ozdemir, 2006)
Carra cheese	50	<100	4.27×10^3	<100	9×10^4	(Aygun *et al*., 2005)
***E. coli* O157**						
Unspecified raw milk cheese	50	<1	-	4.0×10^2	6.0×10^4	(Oksuz *et al*., 2004)

Table 14: *S. aureus* in raw milk cheeses

Cheese Type	No. Samples	Min	Mean	Median	Max	Reference
Mould ripened soft cheese	34	<100	-	-	1.5×10^5	(De Reu *et al*., 2002)
Red smear soft cheese	14	<100	-	-	1.2×10^5	(De Reu *et al*., 2002)
Blue veined cheese	8	<100	-	-	<1000	(De Reu *et al*., 2002)
Semi-hard cheese	2	<100	-	-	<100	(De Reu *et al*., 2002)
Hard cheese	4	<100	-	-	2.7×10^4	(De Reu *et al*., 2002)
Fresh cheese	8	<100	-	-	1.4×10^4	(De Reu *et al*., 2002)
Unspecified raw milk cheese	37	<2	<100	<2	1×10^6	(Sylven, 1998) from (Lindqvist *et al*., 2002)
Carra cheese	50	<100	2.51×10^3	<100	6×10^4	(Aygun *et al*., 2005)
Tetilla cheese	24	-	-	-	6.17×10^1	(Menendez *et al*., 2001)
Turkish Van otlu cheese	50	3.02×10^2	1.26×10^6	-	1.41×10^7	(Kaan Tekinsen and Ozdemir, 2006)

Table 15: Mesophilic bacteria in raw milk cheeses

Cheese Type	No. Samples	Min	Mean	Median	Max	Reference
Unspecified raw milk cheese	50	3.0×10^3	-	3.5×10^6	6.0×10^8	(Oksuz *et al*., 2004)
Carra cheese	50	3.7×10^4	1.87×10^8	10^7	7.9×10^9	(Aygun *et al*., 2005)
Tetilla cheese	24	-	3.98×10^{10}	-	-	(Menendez *et al*., 2001)

Table 16: *Salmonella* spp. in raw milk cheese

Cheese Type	Organism	No. Samples	Min	Max	Reference
Cheddar cheese	*Salmonella* typhimurium phage type 10	21	0.36 (cells/ 100g)	9.3	(D'Aoust, 1985)
Cheddar cheese	*Salmonella* Heidelberg	7	0.36	1.8	(Fontaine *et al*., 1980)

Table 17: *Listeria* spp. in raw milk cheese

Cheese Type	Organism	No. Samples	Min	Max	Reference
Soft and semi-soft cheese	*Listeria monocytogenes*	333	<100	1×10^5	(Loncarevic *et al*., 1995)
Soft cheese	*Listeria* spp.	222	<100	1×10^5	(Zottola and Smith, 1991)

2.2 Raw goat milk cheese

Table 18: Pathogens in raw goat milk cheese

Organisms Isolated	Sample type	Country	Samples	Max Level	Reference
E. coli	Caprino d'Aspromonte	Italy	27	1×10^8	(Caridi *et al*., 2003)
S. aureus	Raw goat milk cheese	Spain	24	8.8×10^3	(Mor-Mur *et al*., 1992)

2.3 Raw sheep milk cheese

Table 19: Pathogens in raw sheep milk cheese

Organisms Isolated	Sample type	Country	mean cell count	Reference
L. monocytogenes	Soft cheese	Austria	2.0×10^2	(Schoder *et al*., 2003)
Coliforms	Orinotyri - fresh	Greece	2.57×10^7	(Prodromou *et al*., 2001)
Coliforms	Orinotyri - 90 days	Greece	4.47×10^4	(Prodromou *et al*., 2001)

3 European Union Rapid Alert Notifications (2003 – 2006)

Table 20: Summary of Rapid alert notifications (2003-2006) (European Food Safety Authority 2006)

Microorganism	Raw Milk	Pasteurised	Unknown	Total
Listeria monocytogenes	6	1	45	52
Escherichia coli	1	1	7	9
Coliforms	0	0	3	3
Staphylococcus aureus	0	0	2	2
Salmonella spp.	2	0	5	7
Brucella spp.	1	0	0	1
Mould	0	0	1	1
Rupture of cold chain	0	0	1	1
Total	**10**	**2**	**64**	**76**

Table 21: European Union rapid alerts by cheese type (2003 - March 2006)

Heat Treatment	Cheese types	*E. coli**	*Salmonella* spp.	*Listeria monocytogenes*	*Brucella* spp.	*Staphylococcus aureus*	Other	Total
Raw	Raw goat milk cheese			2	1			3
	Raw milk Camembert	1						1
	Raw milk cheese**	1		3		1		4
	Roquefort cheese		1					1
	Semi hard raw milk cheese			1				1
Pasteurised	Pasteurised milk cheese	1		1				2
Unknown	Artisanal cheese					1		1
	Camembert/ Brie		1	3			2	6
	Curd	2						2
	Emmentaler cheese			1				1
	Sheep milk cheese			1				1
	Feta cheese			1				1
	Fresh cheese		1					1
	Goat cheese		2	2				4
	Gorgonzola			16				16
	Halloumi cheese			2				2
	Hard cheese	1						1
	Maroilles soft cheese			1				1
	Parmesan cheese					1		1
	Raclette cheese							1
	Ricotta cheese		1					1
	Saint Nectaire cheese			1				1
	Smoked milk cheese			1				1
	Soft cheese	1		4				5
	Tallegio cheese			1				1
	White mould cheese							1
	Other cheese	1		11			3	12
TOTAL		**9**	**7**	**52**	**1**	**3**	**5**	**76**

* Type of *E. coli* not identified

** *L. monocytogenes* and *S. aureus* found in same cheese

Appendix 6: Codex Standards for Cheese

Cheese is described by Codex Alimentarius in its General Standard for Cheese (CODEX STAN A-6-1798, Rev.1-1999, Amended 2003)

Cheese standards also include specifications in relation to descriptions of product, raw materials, permitted ingredients and food additives. Labelling, contaminants and hygiene are also considered.

Table 1: Codex Standards for cheese

Standard Title	Reference
General/Group Standards	
Code of Hygienic Practice for Milk and Milk Products	CAC/RCP 57-2004
Standard for Milk fat Products	CODEX STAN A-2-1973, Rev.1-1999
General Standard for Cheese	CODEX STAN A-6-1978, Rev.1-1999, Amend. 2-2006
Standard for Whey Cheese	CODEX STAN A-7-1971, Rev.1-1999, Amend. 2-2006
General Standard for named variety processed cheese and spreadable processed cheese	CODEX STAN A-8(a)-1978
Standard for Processed Cheese Preparations (Processed Cheese food and processed cheese spread)	CODEX STAN A-8(b)-1978
Group Standard for Unripened Cheese including Fresh Cheese	CODEX STAN 221-2001
Standard for Cheeses in Brine	CODEX STAN 208-1999, Amend.1-2001
Individual Standards	
Standard for Mozzarella	CODEX STAN 262-2007
Standard for Cheddar	CODEX STAN 263-1966, Amend. 1 2007
Standard for Danbo	CODEX STAN 264-1966, Amend. 1 2007
Standard for Edam	CODEX STAN 265-1966, Amend. 1 2007
Standard for Gouda	CODEX STAN 266-1966, Amend. 1 2007
Standard for Havarti	CODEX STAN 267-1966, Amend. 1 2007
Standard for Samsoe	CODEX STAN 268-1966, Amend. 1 2007
Standard for Emmental	CODEX STAN 269-1967, Amend. 1 2007
Standard for Tilsiter	CODEX STAN 270-1968, Amend. 1 2007
Standard for Saint-Paulin	CODEX STAN 271-1968, Amend. 1 2007
Standard for Provolone	CODEX STAN 272-1968, Amend. 1 2007
Standard for Cottage Cheese, Including Creamed Cottage Cheese	CODEX STAN 273-1968, Amend. 1 2007
Standard for Coulommiers	CODEX STAN 274-1969, Amend. 1 2007
Standard for Cream Cheese	CODEX STAN 275-1973, Amend. 1 2007
Standard for Camembert	CODEX STAN 276-1973, Amend. 1 2007
Standard for Brie	CODEX STAN 277-1973, Amend. 1 2007
Extra Hard Grating Cheese	CODEX STAN 278-1978, Amend. 1 2007

APPENDIX 7: Prevalence of microbiological hazards in raw milk

Raw milk contains a heterogeneous microbiological flora which is derived from several sources including the interior of the udder, exterior surfaces of the animals, the environment, milk-handling equipment and personnel. Milking animals may carry a wide range of microorganisms, some of which are human pathogens and they may contaminate raw milk. In addition, the milking procedures, subsequent collection and storage of milk carry the risk of further contamination with, or growth, of intrinsic pathogens.

There is a fair degree of similarity in pathogens detected in raw cow, goat and sheep milk in Australia and as reported in the international literature.

This risk assessment examines the risks presented by a range of the pathogens that may be associated with raw milk, including: *Campylobacter* spp., *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* (EHEC), and *Salmonella* spp.

Data on the prevalence of specific pathogens in raw milk in Australia has been derived from various State testing programs, industry data, and studies published in the literature, and is summarised below:

Organism	Raw milk contamination		
	Cow	Goat	Sheep
C. jejuni	Australian data ND International data ND – 40%	Australian data 1.39% International data ND – 0.04%	International data ND
S. aureus	Australian data 22.9% (CP Staph) International data 9.7 – 100%	Australian data up to 23.3% International data ND – 96.2%	International data 7 – 33.3%
L. monocytogenes	Australian data ND International data 1 – 60%	Australian data ND - 6.8 % International data ND – 5.8%	International data (Found in ewe's raw milk cheese 46%)
E. coli (EHEC)	Australian data 1 – 3 % International data ND – 33.5%	Australian data 7.37% (*E. coli*) International data ND – 16.3%	International data ND – 12.7%
Salmonella	Australian data 6.2% International data ND – 11.8%	Australian data 0.2 % International data ND	International data ND

ND Not detected

The majority of raw milk produced in Australia, with the exception of some goat milk, is destined for further processing *(i.e.* pasteurisation or other heat-treatment). International data has been referred to where Australian data was unavailable.

1 Raw cow milk

Campylobacter spp. may be shed directly in the milk when the animal has clinical or subclinical mastitis due to *Campylobacter* spp. infection, or indirectly through faecal contamination. International data shows the prevalence of *Campylobacter* spp. in raw milk varies between 1-40% (Table 6). Limited Australian survey data is available. No *Campylobacter* spp. have been detected in raw cow milk during surveys undertaken in South Australia during 1996 - 2000 (95 samples) and in a survey of raw cow milk in Western Australia in 2007 (183 samples).

Enterohaemorrhagic *E. coli* (EHEC) including shiga toxin-producing *E. coli* (STEC) and verocytotoxin-producing *E. coli* (VTEC) are often found in the faeces of healthy cattle, sheep and goats. *E. coli* may also be a cause of environmental mastitis and hence be excreted directly into the milk. Pathogenic *E.coli* (incorporating EHEC, VTEC and STEC) have been reported in raw cow milk at a prevalence of up to 33.5%. *E. coli* O157 is a particularly

virulent strain of EHEC and has been isolated from raw cow milk both on farm and from bulk raw milk tankers (Meng *et al.,* 1998; Meng *et al.,* 2001; Desmarchelier and Fegan, 2003). *E. coli* O157 has often been found in many raw milk samples worldwide (ranging in prevalence from 1-6.9%, although it was reported at 33.5% in Malaysia) (Table 8). The incidence of *E. coli* O157 in Canada, USA, Europe and France has been reported as 2.3%, 3.2%, 3.6% and 2.4% respectively (Schlesser *et al*., 2006). EHEC contamination in Australian raw cow milk varies between 1 - 3% (Dairy Australia, 2006).

L. monocytogenes is ubiquitous in the environment and as such may contaminate raw milk during milking. It can also be a cause of mastitis in milking animals and thus be shed directly into raw milk. Raw cow milk is often tested for *L. monocytogenes* and internationally prevalence has been recorded up to 60% (Table 9). *L. monocytogenes* has not been reported as detected in Australian State monitoring programs (Table 2 and Table 3) or in referenced Australian literature (Table 4).

Salmonella spp. can be found in the intestinal tract of most warm and cold blooded animals. In cattle and sheep the bacterium are carried by both healthy and diseased animals and are generally excreted in the faeces, but can be shed through the udder. Contamination of milk is via faecal contamination, but may also be directly through the udder. International data shows prevalence of *Salmonella* spp. in raw cow milk ranging between 0 - 11.8% (Table 10). South Australian data obtained during the period 1996 - 2000 indicate a contamination rate of 3.7% (108 samples) (Table 2), whilst a later survey in Western Australia reported prevalence of 7.65% (Table 3).

S. aureus may be shed into milk as a result of clinical and subclinical cases of mastitis at levels up to 10^5 cfu/ml. Milk usually becomes contaminated via the animal host or food handlers during milking. International data reports that contamination of cow milk varies between 9.7 - 100% (Table 12). South Australia data indicated a contamination rate of 16.35% (104 samples) during the period 1996 - 2000 (Table 2), whilst the Western Australian survey reported prevalence of 26.78% (Table 3).

1.1 Australian data

Table 1: *Salmonella* isolates from raw cow milk, NEPPS data 1983-2004

Organism	Origin and times isolated
S. Agona	Vic 1
S. Anatum	Vic 1
S. Bovismorbificans 24	WA 24
S. Dublin	Vic 12
S. Kiambu	WA 24
S. Mbandaka	WA 1
S. Ohio	NSW 1
S. Typhimurium 13	Vic 1
S. Typhimurium 44	SA 6, Vic 1
S. Typhimurium 135	Vic 2
S. Typhimurium RDNC	NSW 1, Qld 1
S. Zanzibar	Vic 4

Table 2: Summary of data from South Australia testing program (Period 1996 – 2002)

Test	Standard	No. Samples	No. Failures	% Failure
Coliforms	100 org /ml	353	17	4.82
Salmonella spp.	Nil	108	4	3.70
Campylobacte spp.*r*	Nil	95	0	0
Coag +ve Staph	100 org /ml	104	17	16.35
Cryptosporidium parvum	Nil	26	8	30.77
Listeria monocytogenes	Nil	97	0	0

Table 3: Summary of Western Australia raw cow milk survey (2007)

Analysis	Samples	Failures	Prevalence (%)	Count Cfu/ml	Comment
Aerobic Plate count	183	26	14.21	7.7×10^4	
Bacillus cereus	183	0	0	-	
Bacillus Diarrhoeal Toxin	98	0	0	-	
Campylobacter spp.	183	0	0	-	
Clostridium perfringens	183	0	0	-	
Coagulase Positive *Staphylococci*	183	49	26.78	1.6×10^3	
Staph. Enterotoxin	46	0	0	-	
Coliforms	183	52	28.42	2.5×10^4	
EHEC	118	0	0	-	
E. coli	183	93	50.82	2.5×10^4	
L. monocytogenes	183	0	0	-	
Salmonella spp.	183	14	7.65	-	*S. Bovismorbificans* (2) *S. Typhimurium* (1) *S. Give* (3) *S. Kiambu* (8)

Table 4: Surveys from scientific literature in Australia

Organisms Isolated	Samples	% Positive	Reference
L. monocytogenes	600	0	(Anon, 2003b)
L. monocytogenes	150	0	(Ibrahim and Macrae, 1991)

1.2 International data

Table 5: Prevalence of *Brucella* spp. in raw cow milk

Organisms Isolated	Country	Samples	% Positive	Reference
Brucella spp.	Turkey	35	0	(Kasimoglu, 2002)
B. melitensis	Turkey	35	0	(Kasimoglu, 2002)
B. abortus and B. melitensis	Mexico	265	2.3	(Acedo *et al*., 1997)
B. abortus	New Zealand	115	31	(Blair, 1948)

Table 6: Prevalence of *Campylobacter* spp. in raw cow milk

Organisms Isolated	Country	Samples	% Positive	Reference
Campylobacter spp.	US	195	1.5	(Lovett *et al*., 1983)
Campylobacter spp.	US	108	0.9	(Doyle and Roman, 1982)
Campylobacter spp.	Netherlands	200	0	(Oosterom *et al*., 1982)
Campylobacter spp.	US	50	0	(Wyatt and Timm, 1982)
Campylobacter spp.	UK	11 - Farm 5 - Retail 40 - Cow	18.2 - Farm 40 - Retail 5 - Cow	(Hutchinson *et al*., 1985)
Campylobacter spp.	UK	985 - Retail 153 - Farm	5.9 - Retail 5.9 - Farm	(Humphrey and Hart, 1988)
Campylobacter spp.	USA	237	0.42	(McManus and Lanier, 1987)
Campylobacter spp.	UK	111	8.1	(Humphrey and Beckett, 1987)
Campylobacter spp.	Netherlands	904	4.5	(Beumer *et al*., 1988)
Campylobacter spp.	Manitoba	192 - Farm 64 - Dairies	1.56 - Farm 0 - Dairies	(Davidson *et al*., 1989)
Campylobacter spp.	Switzerland	496	0	(Bachmann and Spahr, 1995)
Campylobacter spp.	US	292	12.3	(Rohrbach *et al*., 1992)
Campylobacter spp.	Switzerland	3/83	3.6	(Wegmuller *et al*., 1993)
Campylobacter spp.	UK	1097	1.7	(De Louvois and Rampling, 1998)
Campylobacter spp.	US	131	9.2	(Jayarao and Henning, 2001)
Campylobacter spp.	Canada	1720	0.5	(Steele *et al*., 1997)
Campylobacter spp.	France	69	1.45	(Desmasures *et al*., 1997)
Campylobacter spp.	UK	610	0.8	(Food Standards Agency, 2003)
Campylobacter spp.	Turkey	211	8.1	(Uraz and Yucel, 1999)
Campylobacter spp.	Ireland	62	1.6	(Whyte *et al*., 2004)
Campylobacter spp.	EU	1403	0.21	(European Commission, 2003b)
Campylobacter spp.	US	248	2.2	(Jayarao *et al*., 2006)
Campylobacter spp.	Pakistan	127	10.2	(Hussain *et al*., 2007)
Campylobacter spp.	EU	1431	0.35	(European Commission, 2004)
Campylobacter spp.	US	265	0	(Murinda *et al*., 2004)

Table 7: Prevalence of *Coxiella* spp. in raw cow milk

Organisms Isolated	Country	Samples	% Positive	Reference
C. burnetii	UK	373	21.2 (ELISA)	(Paiba *et al*., 1999)
C. burnetii	Japan	62	33.9 (PCR-ELISA)	(Muramatsu *et al*., 1997)
C. burnetii	Nigeria	169	24	(Adesiyun *et al*., 1985)
C. burnetii	US	109	7.3	(Enright *et al*., 1957)

Table 8: Prevalence of pathogenic *E. coli* in raw cow milk

Organisms Isolated	Country	Samples	% Positive	Reference
E. coli	UK	985 - Retail 153 - Farm	63.4 - Retail 61.4 - Farm	(Humphrey and Hart, 1988)
E. coli O157:H7	USA	23	4.3	(Wells *et al*., 1991)
E. coli O157:H7	USA	115	10	(Padhye and Doyle, 1991)
E. coli O157:H7	UK	329	0	(Mechie *et al*., 1997)
E. coli	USA	77,172	4.4	(Makovec and Ruegg, 2003)
E.coli	Trinidad	287	75.6	(Adesiyun *et al*., 1995)
VTEC	Trinidad	507	4.9	(Adesiyun, 1994)
E. coli O157:H7	USA	603	0	(Hancock *et al*., 1994)
E. coli O157	Trinidad	188	6.9	(Adesiyun *et al*., 1995)
E. coli	France	69	89.8	(Desmasures *et al*., 1997)
E. coli O157	Scotland	500	0	(Coia, 2001)
STEC	USA	131	3.8	(Jayarao and Henning, 2001)
E. coli O157:H7	Netherlands	1,011	0	(Heuvelink *et al*., 1998)
VTEC	Canada	1,720	0.87	(Steele *et al*., 1997)
VTEC	Trinidad	175	9.7	(Adesiyun *et al*., 1997)
E. coli O157:H7	USA	42	0	(Ansay and Kaspar, 1997)
VTEC	Northern Ireland	420	2.14	(McKee *et al*., 2003)
E. coli O157:H7	Italy	100	0	(Massa *et al*., 1999)
E. coli O157:H7	UK	610	0.2	(Food Standards Agency 2003)
E. coli	UK	610	52	(Food Standards Agency 2003)
E. coli O157	Italy	811	0	(Conedera *et al*., 2004)
E. coli O157:H7	USA	268	0.75	(Murinda *et al*., 2002b)
ETEC	Zimbabwe	6	33.3	(Gran *et al*., 2003)
VTEC	EU and Norway	1629	3.4	(European Commission, 2003b)
STEC	USA	248	2.4	(Jayarao *et al*., 2006)
E. coli O157:H7	Costa Rica	100	2	(Reuben *et al*., 2003)
VTEC	EU and Norway	2968	0.7	(European Commission, 2004)
E. coli O157:H7	USA	859	0.6	(Karns *et al*., 2007)
E. coli	UK	-	-	(Hutchison *et al*., 2005)
E. coli O157:H7	Belgium	143	0.7	(De Rue *et al*., 2004)
E. coli O157:H7	Malaysia	930	65 – *E.coli* 33.5 – O157	(Chye *et al*., 2004)
E. coli	Brazil	210	36.8	(Nero *et al*., 2004)
E. coli O157	Turkey	100	1	(Oksuz *et al*., 2004)

Table 9: Prevalence of *Listeria* in raw cow milk

Organism isolated	Country	Samples	% Positive	Reference
L. monocytogenes	Denmark	36199	1.2	(Jensen *et al*., 1996)
L. monocytogenes	US	121 – Milk tanks 14 – Filter socks	12 – M 14 - S	(Hayes *et al*., 1986)
L. monocytogenes	US	650	4.2	(Lovett *et al*., 1987)
L. monocytogenes	Spain	95	45.3	(Rodriguez *et al*., 1985)
L. monocytogenes	Switzerland	4046 340	0.4 0.6	(Bachmann and Spahr, 1995)
L. monocytogenes	USA	200	4	(Liewen and Plautz, 1988)
L. monocytogenes	Finland	314	4.1	(Husu, 1990)
L. monocytogenes	USA	2511	2.9	(Doores and Amelang, 1988)
L. monocytogenes	Canada	315	5.4	(Slade *et al*., 1988)
L. monocytogenes	Canada	445	1.3	(Farber *et al*., 1988b)
L. monocytogenes	Ireland	113	5.3	(Harvey and Gilmour, 1992)
L. monocytogenes	Australia	600	0	(Anon, 2003b)
L. monocytogenes	Scotland	180	1.0 – 3.8	(Fenlon and Wilson, 1989)
L. monocytogenes	Germany	201	0	(Eliskases-Lechner and Ginzinger, 1999)
L. monocytogenes	Canada	192 - Farm 64 - Diaries	1.04 3.13	(Davidson *et al*., 1989)
L. monocytogenes	France	2000	3.2	(Sanaa *et al*., 1993)
L. monocytogenes	USA	300	3	(Lund *et al*., 1991)
L. monocytogenes	Canada	-	-	(Fedio *et al*., 1990)
L. monocytogenes	Canada	36 - Tankers 36 – Tankers 426 – Bulk vat	2.8 - T 11.1 - T 1.9 - B	(Fedio and Jackson, 1990)
L. monocytogenes	Australia	150	0	(Ibrahim and Macrae, 1991)
L. monocytogenes	USA	292	4.1	(Rohrbach *et al*., 1992)
L. monocytogenes	Japan	943 – Bulk vat 504 - Farm	0.32 - B 28.6 - F	(Yoshida *et al*., 1998b)
L. monocytogenes	Japan	51	50.9	(Yoshida *et al*., 1998a)
L. monocytogenes	Canada	20 – Bulk vat 401 - Cow	60 – B 5.2 - C	(Fedio and Jackson, 1992)
L. monocytogenes	England and Wales	2009	5.1	(O'Donnell, 1995)
L. monocytogenes	Scotland	640	6.6	(Fenlon *et al*., 1995)
L. monocytogenes	Sweden	294 – Bulk vat 295 - Silo	1.0 - B 19.6 - S	(Waak *et al*., 2002)
L. monocytogenes	USA	131	4.6	(Jayarao and Henning, 2001)
L. monocytogenes	Canada	1,720	2.7	(Steele *et al*., 1997)
L. monocytogenes	France	69	5.8	(Desmasures *et al*., 1997)
L. monocytogenes	France	1459	2.4	(Meyer-Broseta *et al*., 2003)
L. monocytogenes	Spain	774	3.62	(Gaya *et al*., 1998)
L. monocytogenes	USA	404	12.6	(Hassan *et al*., 2000)
L. monocytogenes	Turkey	100	4	(Vardar-Unlu *et al*., 1998)

Table 9 cont: Prevalence of *Listeria* in raw cow milk

Organism isolated	Country	Samples	% Positive	Reference
L. monocytogenes	Mexico	1300	13	(Carlos *et al*., 2001)
L. monocytogenes	UK	610	17	(Food Standards Agency 2003)
L. monocytogenes	Brazil	12	8.3	(Silva *et al*., 2003)
L. monocytogenes	Turkey	2/211	0.94	(Uraz and Yucel, 1999)
L. monocytogenes	USA	474 474 25	4.9 7.0 68	(Muraoka *et al*., 2003)
L. monocytogenes	Czech Republic	278	2.1	(Navratilova *et al*., 2004)
L. monocytogenes	EU and Norway	1377	1.3	(European Commission, 2003b)
L. monocytogenes	USA	248	1.2	(Jayarao *et al*., 2006)
L. monocytogenes	USA	861	6.5	(Van Kessel *et al*., 2004)
L. monocytogenes	Slovak Republic	25	20	(Holko *et al*., 2002)
L. monocytogenes	Belgium	143	6.3	(De Rue *et al*., 2004)
L. monocytogenes	USA	860	6.5	(USDA/APHIS, 2003)
L. monocytogenes	Brazil	210	0	(Nero *et al*., 2004)
L. monocytogenes	Portugal	105	1.9	(Kongo *et al*., 2006)
L. monocytogenes	Costa Rica	100	3.0	(Reuben *et al*., 2003)
L. monocytogenes	Malaysia	930	1.9	(Chye *et al*., 2004)
L. monocytogenes	Turkey	47	0	(Aygun and Pehlivanlar, 2006)

Table 10: Prevalence of *Salmonella* spp. in raw cow milk

Organism	Country	Samples	% Positive	Reference
Salmonella spp.	UK	985	0.2	(Humphrey and Hart, 1988)
Salmonella spp.	Canada	1140	2.5	(McEwen *et al*., 1988)
Salmonella spp.	USA	678	4.7	(McManus and Lanier, 1987)
Salmonella spp.	Germany	201	0	(Eliskases-Lechner and Ginzinger, 1999)
Salmonella spp.	England & Wales	1,673	0.36	(O'Donnell, 1995)
Salmonella spp.	USA	292	8.9	(Rohrbach *et al*., 1992)
Salmonella spp.	Switzerland	456	0	(Bachmann and Spahr, 1995)
Salmonella spp.	Canada	1,720	0.17	(Steele *et al*., 1997)
Salmonella spp.	USA	131	6.1	(Jayarao and Henning, 2001)
Salmonella spp.	France	69	2.9	(Desmasures *et al*., 1997)
Salmonella spp.	USA	404	1.5	(Hassan *et al*., 2000)
Salmonella spp.	UK	610	0.3	(Food Standards Agency 2003)
Salmonella spp.	Ireland	29	3.4	(Food Safety Authority of Ireland, 2004)
Salmonella spp.	USA	248	6.0	(Jayarao *et al*., 2006)
Salmonella spp.	USA	268	2.2	(Murinda *et al*., 2002a)
Salmonella spp.	USA	861	2.6	(Van Kessel *et al*., 2004)
Salmonella spp.	USA	854	11.8	(Karns *et al*., 2005)
Salmonella spp.	USA	860	2.7	(USDA/APHIS, 2003)
Salmonella spp.	Brazil	210	0	(Nero *et al*., 2004)
Salmonella spp.	Belgium	143	0	(De Rue *et al*., 2004)
Salmonella spp.	Malaysia	930	1.4	(Chye *et al*., 2004)

Table 11: *Salmonella* spp. isolates from raw milk, French National Research Council data 1990-1997

Organism – Decreasing Order
S. Typhimurium
S. Montevideo
S. Indiana
S. Anatum
S. Enteritidis
S. Kottbus
S. Dublin

Table 12: Prevalence of *S. aureus* in raw cow milk

Organisms Isolated	Country	Samples	% Positive	Reference
S. aureus	Norway	220	75	(Jorgensen *et al*., 2005a)
S. aureus	US	118	60	(Sato *et al*., 2004)
S. aureus	Denmark	40	55	(Sato *et al*., 2004)
S. aureus	Malaysia	930	>60	(Fook *et al*., 2004)
S. aureus	USA	77,172	9.7 - 17.7	(Makovec and Ruegg, 2003)
S. aureus	Czech Republic	111	34.2	(Schlegelova, 2002)
S. aureus	Canada	21	90.4	(Tondo *et al*., 2000)
S. aureus	Italy	794	34.3	(Moretti *et al*., 1998)
S. aureus	France	69	62	(Desmasures *et al*., 1997)
S. aureus	Brazil	19	57.9	(De Gomes and Gallo, 1995)
S. aureus	Denmark	4,645	10.2	(Aarestrup *et al*., 1995)
S. aureus	Trinidad	287	100	(Adesiyun *et al*., 1995)

2 Raw goat milk

There is limited published microbiological data on Australian raw goat milk, however, international data indicate raw goat milk may contain *Aeromonas* spp., *Brucella* spp., *Campylobacter* spp., pathogenic *E. coli, L. monocytogenes*, *Mycobacterium* spp., *S. aureus* and *Yersinia enterocolitica.* In Australian surveys, potential pathogens detected in raw goat milk have included *E. coli*, *L. monocytogenes*, and *Y. enterocolitica*.

In the literature, *Campylobacter* spp. have been isolated from raw goat milk with very low prevalence (0.04%) in the UK and although it was identified in Switzerland, no prevalence was reported (Table 19). Western Australia has been the only state to detect *Campylobacter* spp. (during the period 2003 – 2006) in raw goat milk with 6 out of 113 samples (5.3%) testing positive (Table 14). *Campylobacter* spp. have not been isolated during any other monitoring program in Australia.

The prevalence of *E. coli* (both generic and pathogenic) in raw goat milk has been cited as ranging between 0 - 16.3 % internationally. STEC was isolated in a Swiss study at a prevalence of 16.3% and EHEC was reported at a prevalence of 0.7% in the UK (Muehlherr *et al.*, 2003). European data shows pathogenic *E. coli* to be found in 0 – 16.3% of raw goat milk (Table 21). An Italian study also reported the prevalence of *E. coli* O157:H7 at 1.7%. (Table 21). No Australian data is available for STEC or EHEC;

however generic *E. coli* prevalence is reported as being 7.37% (Table 16). It should be highlighted that a recent routine sample of raw goat milk was reported as testing positive for Shiga-like toxin (SLTEC) during routine testing in Western Australia (pers. comm. Calder, 2008).

L. monocytogenes was detected at levels up to 2.56% (Spain) (Table 22). In a pilot study in New South Wales in 2002, no *L. monocytogenes* was found (Table 15). In the early 1990's, *L. monocytogenes* had been detected at very low levels (1.4%) (Table 17), however no *Listeria* has been detected under State monitoring programs since 1993 (Table 14).

While goat milk is often tested for the presence of *Salmonella* spp., it is rarely detected (Table 23). *Salmonella* spp. have been reported in Australia during the 1970's at prevalence of 0.34% (Table 17) and in raw goat milk in New South Wales during the period 1993 - 1999 in one out of two samples. Data collated by the National Enteric Pathogen Surveillance Scheme (NEPPS) from 1983 - 2004 showed that of the 1,156 dairy samples positive for *Salmonella* spp., only 14 isolates were detected from raw goat milk (Table 13). Australian data since 1993 indicates an overall contamination rate of 0.2% (Table 16).

Prevalence of *S. aureus* contamination varies greatly with between 0 - 96.2% of international samples tested being found positive (Table 24). Coagulase positive *Staphylococcus* spp. have been detected in goat milk in all States of Australia except Queensland, with an overall contamination rate of 20.32% (Table 16).

2.1 Australian data

Table 13: *Salmonella* spp. isolates from raw goat milk, NEPPS data 1983-2004.

Organism	Origin and times isolated
S. Anatum	NSW 1, Qld 1
S. Choleraesuis bv Kunzendorf Australia	WA 7
S. Saintpaul	NSW 3
S. subsp IIIb ser 61:l,v:z35	Qld 2

Table 14: Summary of data from State testing programmes (1993 - 2006)

State	*Campylobacter*	*Coag + Staph*	*Coliforms*	*E. coli*	*Listeria* spp.	*Salmonella* spp.
NSW 93 – 99*	-	5.9% *(2/34)*	17.2% *(17/99)*	2% *(1/51)*	-	50% *(1/2)*
NSW 02 – 05*	0% *(0/263)*	12.8% *(34/266)*	-	10.5% *(28/266)*	0% *(0/266)*	0% *(0/266)*
SA 95 – 01**	0% *(0/38)*	7.9% *(3/38)*	9.5% *(26/274)*	-	0% *(0/38)*	0% *(0/38)*
SA 00 – 05**	-	34.1% *(30/88)*	-	12.5% *(3/24)*	0% *(0/77)*	0% *(0/77)*
QLD 03 – 06#	0% *(0/19)*	0% *(0/24)*	1.5% *(1/65)*	0% *(0/39)*	0% *(0/21)*	0% *(0/21)*
WA 03 – 06##	5.3% *(6/113)*	21.6% *(24/111)*	31% *(38/122)*	4% *(5/122)*	0% *(0/120)*	0% *(0/107)*

* NSW Food Authority, ** Dairy Authority of South Australia, # Safefood Queensland, ## Department of Health Western Australia

Table 15: Summary of outcomes of testing data from SA risk assessment (Pointin *et al.*, 2004)

State	*Campylobacter*	*Coag + Staph*	*Coliforms*	*E. coli*	*Listeria* spp.	*Salmonella* spp.	*Y. enterocolitica*
NSW 1972 (survey)	-	5.6% *(4/72)*	34.7% *(25/72)*	-	-	-	-
NSW 2001 (survey)	-	23.3% *(14/60)*	-	21.7% *(13/60)*	0% *(0/60)*	0% *(0/60)*	1.7% *(1/60)*
NSW 2002 (pilot study)	0% *(0/59)*	-	-	20.4% *(12/59)*	6.8% *(4/59)** 0% *(0/59)#*	0% *(0/59)*	0% *(0/59)*
SA 1995 – 2003 (testing)	0% *(0/79)*	12% *(10/81)*	7% *(26/392)*	-	0% *(0/79)*	0% *(0/79)*	0% *(0/54)*

* *L. innocua*, # *L. monocytogenes*

Table 16 Overall combined prevalence of pathogens in Australia (complied from State testing data)

Period	*Campylobacter*	*Coag + Staph*	*Coliforms*	*E. coli*	*Listeria* spp.	*Salmonella* spp.
1993 - 2006	1.39%	20.32%	14.64%	7.37%	0%	0.2%

Table 17: Surveys from scientific literature in Australia

Organisms Isolated	Sampling period	% Positive	Reference
B. cereus	Aug - Dec 1978	6.9% (20/291)	(Jensen and Hughes, 1980)
E. coli	Aug - Dec 1978	60.5% (176/291)	(Jensen and Hughes, 1980)
Salmonella spp.	Aug - Dec 1978	0.34% (Level=3.44E-05)	(Jensen and Hughes, 1980)
S. aureus	Aug - Dec 1978	5.5% (16/291)	(Jensen and Hughes, 1980)
S. aureus		<1% (<8/896)	(Ryan and Greenwood, 1990)
L. monocytogenes		1.4% (9/69)	(Arnold and Coble, 1995)
Y. enterocolitica	Aug - Dec 1978	12.8% (35/274)	(Hughes and Jensen, 1981)

2.2 International data

Table 18: Prevalence of *Brucella* spp. in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
B. abortus	Mexico	24	6.4	(Acedo *et al.*, 1997)
B. melitensis	Mexico	24	8.4	(Acedo *et al.*, 1997)

Table 19: Prevalence of *Campylobacter* spp. in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
Campylobacter spp.	UK	NA	0.04	(Burden, 1989)
Campylobacter spp.	Switzerland	344	0	(Muehlherr *et al*., 2003)
Campylobacter spp.	UK	100	0	(Little and De Louvois, 1999)

Table 20: Prevalence of *Coxiella* spp. in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
Coxiella burnetii	US	29	7	(Ruppanner *et al*., 1978)

Table 21: Prevalence of *E. coli* in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
STEC	Switzerland	344	16.3	(Muehlherr *et al*., 2003)
EHEC	UK	94	0.7	(Anon, 1999)
E. coli	UK	2462	10	(Roberts, 1985)
E. coli	USA	2911	1.6	(White and Hinckley, 1999)
E coli	Austria	204	1.5	(Pernthaner *et al*., 1993)
E. coli O157:H7	Italy	60	1.7	(Foschino *et al*., 2002)
E. coli O157:H7	UK	100	0	(Little and De Louvois, 1999)

Table 22: Prevalence of *Listeria* spp. in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
L. monocytogenes	UK	100	0	(Little and De Louvois, 1999)
L. monocytogenes	India	64	1.56	(Barbuddhe *et al*., 2000)
L. monocytogenes	UK	94	2.09	(Anon, 1999)
L. monocytogenes	Spain	1445	2.56	(Gaya *et al*., 1996)
L. monocytogenes	USA	450	3.8	(Abou-Eleinin *et al*., 2000)
L. monocytogenes	Portugal	39	0	(Guerra *et al*., 2001)
L. innocua	Spain	1445	1.73	(Gaya *et al*., 1996)
L. innocua	USA	450	5.8	(Abou-Eleinin *et al*., 2000)
Listeria spp.	Portugal	39	5	(Guerra *et al*., 2001)

Table 23: Prevalence of *Salmonella* spp. in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
Salmonella spp.	Spain	1445	0	(Gaya *et al*., 1996)
Salmonella spp.	Europe	50	0	(Abo-Elnaga *et al*., 1985)
Salmonella spp.	UK	2463	0	(Roberts, 1985)
Salmonella spp.	Switzerland	344	0	(Muehlherr *et al*., 2003)
Salmonella spp.	Italy	60	0	(Foschino *et al*., 2002)
Salmonella spp.	UK	100	0	(Little and De Louvois, 1999)
Salmonella spp.	Bulgaria	60	0	(Vashin *et al*., 1999)

Table 24: Prevalence of *Staphylococcus* spp. in raw goat milk

Organisms Isolated	Country	Samples	% Positive	Reference
S. aureus	Europe	50	0	(Abo-Elnaga *et al*., 1985)
S. aureus	US	2911	11	(White and Hinckley, 1999)
S. aureus	France	238	2	(De Buyser *et al*., 1987)
S. aureus	Norway	213	96.2	(Jorgensen *et al*., 2005b)
S. aureus	Switzerland	344	31.7	(Muehlherr *et al*., 2003)
S. aureus	Italy	60	43	(Foschino *et al*., 2002)
S. aureus	Austria	359	17.6	(Deinhofer and Pernthaner, 1995)
S. aureus	UK	2,493	4	(Roberts, 1985)
S. aureus	UK	100	15	(Little and De Louvois, 1999)
S. aureus	Greece	1350	10	(Kalogridou-Vassiliadou, 1991)
S. aureus	Iraq	297	3	(Al-Graibawi *et al*., 1986)
Coag -ve staph	Austria	204	55	(Pernthaner *et al*., 1993)
Coag +ve staph	Austria	204	37.3	(Pernthaner *et al*., 1993)
Staphylococcus spp.	Austria	204	1.5	(Pernthaner *et al*., 1993)

3 Raw sheep milk

There is little data available on the prevalence of pathogens in raw sheep milk. International data suggests that prevalence of *S. aureus*, *Brucella spp.* and *E. coli* (EHEC) ranges from 7 – 33.3%, 14.2 - 46%, and 1 – 12.7% respectively (Table 29, Table 25 and Table 27). Surveys for *C. jejuni* and *Salmonella* failed to detect these organisms in sheep milk (Table 26 and Table 28).

3.1 International data

Table 25: Prevalence of *Brucella* spp. in raw sheep milk

Organisms Isolated	Country	Samples	% Positive	Reference
Brucella spp.	Turkey	35	14.2	(Kasimoglu, 2002)
Brucella spp.	Italy	46	46	(Tantillo *et al*., 2001)

Table 26: Prevalence of *Campylobacter* spp. in raw sheep milk

Organisms Isolated	Country	Samples	% Positive	Reference
Campylobacter spp.	Switzerland	63	0	(Muehlherr *et al*., 2003)
Campylobacter spp.	UK	26	0	(Little and De Louvois, 1999)

Table 27: Prevalence of *E. coli* in raw sheep milk

Organisms Isolated	Country	Samples	% Positive	Reference
E. coli O157	Italy	502	0	(Conedera *et al*., 2004)
E. coli O157:H7	UK	26	0	(Little and De Louvois, 1999)
STEC	Switzerland	63	12.7	(Muehlherr *et al*., 2003)

Table 28: Prevalence of *Salmonella* spp. in raw sheep milk

Organisms Isolated	Country	Samples	% Positive	Reference
Salmonella spp.	Switzerland	63	0	(Muehlherr *et al*., 2003)
Salmonella spp.	UK	26	0	(Little and De Louvois, 1999)

Table 29: Prevalence of *Staphylococcus* spp. in raw sheep milk

Organisms Isolated	Country	Samples	% Positive	Reference
S. aureus	Switzerland	63	33.3	(Muehlherr *et al*., 2003)
S. aureus	UK	126	7	(Little and De Louvois, 1999)

APPENDIX 8: Risk assessment – Extra hard raw milk cheeses

1 Introduction

Cheese can be categorised in a variety of ways, but more traditional characterisation schemes are based principally on the rheological properties of the cheese, using terminology such as extra hard, hard, semi-hard/soft or soft. There is some correlation between these descriptors and the moisture content of cheese, with extra hard cheeses characterised by a moisture content of less that 35%, and hard cheeses between 36 - 39% moisture. For a detailed description of cheese categories, cheese manufacture and technology refer to Appendix 3.

The extra hard cheeses include the well known Italian parmesan style cheeses, such as Parmigiano Reggiano and Grana Padano, Romano, Asiago and Montasio , as well as varieties produced in Spain and Russia and the Swiss produced Sbrinz cheese. Extra hard varieties can be manufactured from cows', sheep's or goats'milk or mixtures thereof. These cheeses are typically prepared using relatively high curd-cooking temperatures (>45ºC) and long storage/maturation times (8 - 24 months), resulting in low moisture contents, generally less than 35%. The majority of the extra hard cheese varieties such as Parmigiano Reggiano and Grana Padano (parmesan style cheeses) originated in Italy and are designated Denominazione d'origine controllata (DOC)[31]. These cheeses continue to be manufactured in Italy according to traditional methods using raw milk under strict manufacturing protocols.

Because of their hard texture and strong flavour, extra hard cheeses are generally used in small quantities as grated cheese and are often referred to as extra hard grating cheeses. The Codex standard for *extra hard grating cheese*[32] contains details on the principal characteristics of this class of cheese (such as appearance, texture and origin of milk), and specifies a maximum moisture content of 36% and a minimum period of maturation/curing of not less than 6 months. Whether using traditional techniques or modern manufacturing protocols, there are characteristic steps in the manufacturing of extra hard cheeses, which determine the nature of these cheeses.

This risk assessment qualitatively examines the fate of *Campylobacter jejuni*, Enterohaemorrhagic *Escherichia Coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* during the manufacture of raw milk Parmigiano Reggiano, Grana Padano, Romano, Asiago, Montasio and Sbrinz extra hard cheeses. The information contained in this risk assessment is based on work previously undertaken by FSANZ during the evaluation of Proposal P263 – Safety assessment of raw milk very hard cooked-curd cheeses[33] and Application A357 – Swiss Raw Milk Cheeses[34].

A qualitative framework developed by Food Science Australia was subsequently used to rate the risk to public health and safety from the consumption of raw milk extra hard cheese made from either cow, goat or sheep milk containing these microbiological hazards.

The specific manufacturing processes assessed for Pecorino Romano, Asiago and Montasio cheeses included thermal treatment of the milk (thermisation and pasteurisation). While the

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31 Protected Denomination of Origin (DOC) is a regulated and controlled qualification used within Europe for a number of products including olive oils, wines and cheeses. DOC regulates the area of production and the production system which is considered to make the product unique.

32 Codex International Standard for Extra Hard Grating Cheese, *CODEX STAN C-35-1978.*

33 http://www.foodstandards.gov.au/_srcfiles/P263rawcheeseFAR.pdf

34 http://www.foodstandards.gov.au/_srcfiles/A357%20FAR.pdf

significant effect that thermal treatment would have on the pathogens was noted, the risk assessment analysed the effect of the curd cooking processes, and the effects of ripening and storage on bacterial reduction. This allowed for an evaluation of the production processes on pathogen survival for these cheese types if the raw milk used was not subject to thermisation or pasteurisation. For simplicity, the cheeses will be referred to as Romano, Asiago and Montasio.

2 Hazard identification and hazard characterisation

In evaluating the safety of extra hard raw milk cheeses, the following pathogens were considered: *Campylobacter jejuni/coli*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus*.

A detailed characterisation of these hazards is attached as Appendix 14.

3 Exposure assessment

The extra hard cheeses are hard, grainy cheeses, which are cooked but not pressed during their preparation and aged for periods of up to 2 years. The main generic steps in the making of extra hard cheeses are outlined and described in Figure 1.

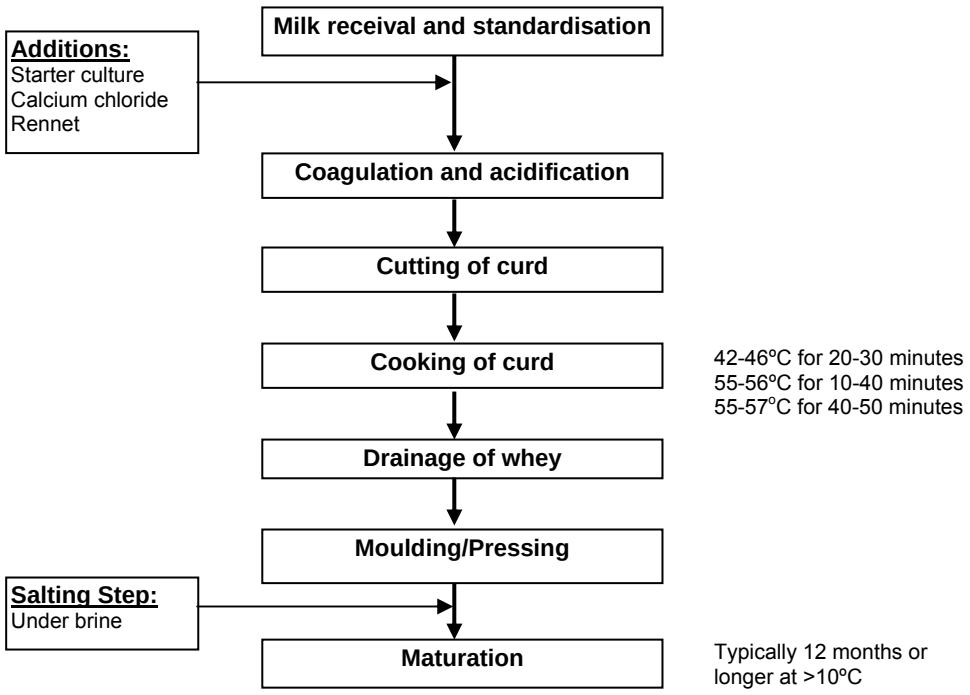

Figure 1: Overview of major steps in the manufacture of extra hard cheese

The actual steps in the preparation of extra hard cheese vary significantly between the individual styles, as described in Table 1.

Table 1: Summary of processing steps for individual extra hard raw milk cheeses

Processing step	Parmigiano Reggiano	Grana Padano	Pecorino Romano	Asiago	Montasio	Sbrinz
Raw milk handling	Cow milk partially skimmed Held for 10 - 12h Whole milk then added next morning	Cow milk partially skimmed Held at 16 - 17°C for 6 - 8h pH 6.4 - 6.7	Sheep milk filtered Stored 4°C ≤ 24h	Cow milk partially skimmed Whole milk added next morning	Whole cow milk	Whole cow milk
Heat treatment	nil	nil	65 - 68°C	73 - 75°C, 15 - 30 sec	73 - 75^{0}C, 15 - 30 sec	nil
Acidification/ Whey starter added	Natural whey starter Mainly lactobacilli	Natural whey starter Mainly lactobacilli 33^{0}C pH 6.2 - 6.5	Thermophilic lactobacillus and streptococcus 38 - 40°C/30 min pH <3.5	Natural whey starter 32 - 36°C	Natural whey starter 35°C pH 6.5 - 6.6 before pH 6.4 after addition	Natural culture in whey Milk warmed to 30 - 32^{o}C for 20 - 30 min
Coagulation/ rennet addition	33 -34°C 10 - 12 min	33°C 10 - 15 min		35°C 20 - 30 min	35°C 20 min	40 min
Curd cutting	43 - 44°C/3 - 4 min				42 - 43°C	
Curd cooking	55 - 56°C/15 - 20 min	55 - 56°C/20 min	45 - 48°C/10 min	42 - 46°C/20 - 30 min	48°C/30 - 40 min	55 - 57^{o}C for 40 - 50 min
Curd resting/ hooping/ pressing	Under whey at 55 - 56°C/45 - 65 min (minimum 40 min) Pressing 3d pH 5.0 - 5.3	Under whey at 55 - 56°C/35 - 45 min (minimum 40 min) Drain for 8h in wooden mould then 2 - 3d in s/s mould at 18 - 20°C	Under whey 45 - 48°C/30 min (minimum)	Out of whey 12h	Out of whey Pressing 3d	Mechanical pressing
Salting	25 - 27d	Brine 22 -2 6% at 15 - 18°C for 25 - 32d	Brine 23 - 24% for 6 - 10d or dry salting 3 - 4 times over 50 - 70d	Brine 20 - 22% at 15°C for 5d pH 5.35	Brine 20 - 22% at 15°C for 5d pH 4.95	Brining 4 - 20 days at 12 - 14^{o}C
Drying	15 - 18°C for 3d					
Ripening	16 - 18°C at 85% relative humidity for 18 - 24 months	16 - 22°C for 14 - 18 months	Minimum of 5 months	15 - 16°C for 3 - 12 months (short) or 9 - 12 months 8 - 9°C (long - Asiago D'Allevo)	15 - 18°C for 3 - 12 months	16 - 18^{o}C for 20 - 35 days 100 - 130 days 12^{o}C Regularly turned Warehouse further stored: 35 - 70 days 14 - 16^{o}C followed by 14 - 18 months at 12^{o}C

The variations in manufacture described in Table 1 result in variations in the intrinsic properties of each cheese type, and influence the extent to which pathogenic microorganisms are eliminated or controlled. The resulting extra hard cheese has varying chemical composition and characteristics (Table 2).

Table 2: Characteristics of selected extra hard raw milk cheeses:

Property	Parmigiano Reggiano	Grana Padano	Pecorino Romano	Asiago	Montasio	Sbrinz
pH	5.4 - 5.5	5.4 - 5.6	NA	5.35	5.0 - 5.4	5.4
Moisture (%)	30.76	32.46	32 (5 months)	34	32	31 - 33
NaCl (g/100g)	1.3	1.4	5.0	NA	NA	1.9

3.1 Survival of pathogens during the manufacture of extra hard cheeses

3.1.1 *Campylobacter jejuni* and *Campylobacter coli*

C. jejuni/*coli* have highly specific growth requirements and are extremely sensitive to sub-optimal environmental conditions. Even if these organisms are present in the raw milk used for the production of extra hard cheeses, they will not grow unless the temperatures during storage and transport are greater than 30°C and microaerophilic conditions of reduced $O_2$and increased CO_2 are provided (Park, 2002).

Sheep milk used in Pecorino Romano cheese and cow milk used in Grana Padano cheese are held at 4°C and 16 - 17°C, respectively, and *C. jejuni*/*coli* will not grow at these temperatures. Microaerophilic conditions will not prevail; hence growth is unlikely, although these organisms will survive these storage temperatures.

C. jejuni/*coli* are thermophilic, however, they are sensitive to heat and readily inactivated by correct application of pasteurisation and cooking processes (Park, 2002). *C. jejuni* has a D-value of 0.7-1.0 minutes at 55°C in skim milk (Doyle and Roman, 1982). Thermisation at 65 - 68°C for sufficient time or pasteurisation (73 - 75°C for 15 - 30 sec) will result in more than a 5 log reduction in thermophilic *Campylobacter* spp. (D'Aoust *et al*., 1988).

During the production of extra hard cheeses, raw milk is acidified and set by the addition of a starter culture and rennet. The starter for all assessed extra hard cheeses, except Pecorino Romano, is aspirated from the whey fermentation of the previous day and is composed mainly of thermophilic lactobacilli. The starter for Pecorino Romano is a thermophilic lactobacilli and streptococci culture. The temperature of the milk is initially increased to 33 - 40°C for 10 - 30 minutes to encourage the growth of the starter culture. While these temperatures may be suitable for growth of thermophilic *Campylobacter* spp. other strict conditions required for growth are not met such as an elevated level of CO_2 (10%) and reduced level of O_2 (~5 - 6%) (ICMSF, 1996).

Raw milk used for production of Parmigiano Reggiano, Grana Padano and Sbrinz receive no thermal pre-treatment, with curd cooked at higher temperatures *e.g.* 55-56°C for 15 - 20 minutes and 55 - 57^{o}C for 40 - 50 minutes. The curd made from thermised sheep milk (Pecorino Romano) is cooked at 45 - 48°C for 10 minutes and is held at this temperature for up to 30 minutes while the curd is pressed under the whey. When pasteurised milk is used in the manufacture of cheeses, *i.e.* Asiago and Montasio, a lower curd cooking temperature is used, 42 - 46°C for 20 - 30 minutes and 48°C for 30 - 40 minutes, respectively.

The curd of Parmigiano Reggiano and Grana Padano is allowed to rest in contact with the whey in the cooking vat before pressing and hooping so that the period of time at 55-56°C is a minimum of 40 minutes. In skim milk *C. jejuni* has a D-value of 7.2 - 12.8 minutes at 48°C, 0.7 - 1 minutes at 55°C (Doyle and Roman, 1982) and at 40ºC with optimum gas

atmosphere death occurred (ICMSF, 1996). *C. jejuni* would not survive the curd cooking temperatures used in the manufacture of these hard cheeses. At low curd cooking temperatures *i.e.* <48°C for 30 minutes such as for Pecorino Romano, a 2.5 log reduction of thermophilic *Campylobacter* spp. may be achieved.

Campylobacter survive poorly in mildly acidic environments and in the presence of 2% or more salt, and fails to grow at water activities of less than 0.987 (ICMSF, 1996). The salt concentration of extra hard cheeses is 1.4g/100g (Parmigiano Reggiano), 1.5g/100g (Grana Padano), 1.9g/100g (Sbrinz) and 5g/100g (Pecorino Romano). The final pH of these cheeses is generally less than 5.5 (Grana Padano: 5.4 - 5.6; Asiago: 5.35; Sbrinz: 5.4; and Montasio: 5.4). After brining, ripening and storage, the conditions in the cheese are expected to be lethal to *C. jejuni/coli*.

Overwhelming, the available data demonstrates that *C. jejuni/coli* are unlikely to be a hazard in correctly manufactured extra hard cheeses. This is due to thermal processing applied during pre-treatment of milk and/or curd cooking inactivating *Campylobacter* spp. present in the raw milk and conditions in the cheese after fermentation and during ripening inactivating any survivors.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
All extra hard raw milk cheeses	Unlikely to grow due to falling pH. Curd cooking in Parmigiano Reggiano, Grana Padano and Sbrinz is lethal to *Campylobacter* spp.	Do not survive mildly acidic conditions or water activity <0.987.	Eliminates

3.1.2 *Escherichia coli* (EHEC)

E. coli grow over a temperature range of 7 - 46°C, with optimum growth temperatures being 35 - 40°C (ICMSF, 1996). Any *E. coli* present in the raw milk will grow if the temperature during storage and transport are within this range with the amount of growth dependent on the duration and temperature of storage.

The raw milk used in many extra hard cheeses is partially skimmed after storage, which allows a cream layer to rise to the surface of the milk. For Grana Padano cheese, milk is partially skimmed after 6 - 8 hours storage at 16 - 17°C. During this time, growth of *E. coli* may occur although it is likely that the bacteria may remain in the lag phase of growth . Sheep milk for Pecorino Romano is stored at 4°C and this will inhibit the growth of *E. coli*, although it will survive. Specific details on storage temperatures for milk used to produce Montasio, Asiago, Parmigiano Reggiano and Sbrinz were not available. Regardless of the actual conditions, it can be assumed that no more than 1 log increase in numbers is possible during the transportation and storage of milk prior to cheesemaking.

E. coli are sensitive to heat and readily inactivated by heat treatments such as thermisation or pasteurisation of milk (ICMSF, 1998). Thermisation processes such as heating to 63 - 65°C for 15 - 20 seconds (IDF definition) is likely to result in a 2 - 5 log reduction in *E. coli* (D'Aoust *et al*., 1988; Morgan *et al*., 1988). The D-value at 64°C is 3 - 9.6 sec. Pasteurisation at 73 - 75°C for 15 - 30 seconds will eliminate pathogenic *E. coli*.

Milk used for the manufacture of extra hard cheeses is heated to 30 - 40°C to encourage the growth of the starter culture and facilitate acid production. Despite the presence of large numbers of starter organisms, *E. coli* has been shown to multiply in milk with added starter culture (ICMSF, 1996). At a pH of 5.1 - 6.6 and at temperatures of 24 - 36ºC, a 1 - 2.5 log increase in numbers may occur over 5 - 7 hours. At 32.8 - 40°C and pH of 6.1 - 6.6, a 3 log increase occurred over 3.5 hours. While the temperature and the pH of the milk may allow growth, the period of holding under these conditions is relatively brief, and it is unlikely to result in significant growth of *E. coli*.

For cheeses exposed to high curd cooking temperatures, this stage has a major impact on any *E. coli* that have survived initial processing or been introduced into the curd. The temperature limit for growth of *E. coli* is 46°C with some strains of *E. coli* O157 (EHEC) unable to grow above 42.5°C in selective media (Desmarchelier and Grau, 1997). The pasteurised milk cheeses of Asiago and Montasio are exposed to relatively low curd cooking temperatures of 42 - 46°C for 20 - 30 minutes and 48°C for 30 - 40 minutes respectively. At these temperatures growth of *E. coli* could occur, however the raw milk has been pasteurised so no *E. coli* should be present at this step.

Curd made from thermised milk *i.e.* Pecorino Romano, is cooked at 45 - 48°C for 10 minutes and is held at this temperature up to 30 minutes while the curd is pressed under the whey. While the growth of *E. coli* will be inhibited at this temperature, there is limited further inactivation of the *E. coli*. Thermisation of the milk will have resulted in a 2 - 5 log reduction of *E. coli*.

Raw milk used for production of Parmigiano Reggiano and Grana Padano is not thermally treated; however, the curd is generally cooked at high temperatures *e.g.* 55 - 56°C for 15 - 20 minutes, plus the curd is rested in the whey for a further 45 - 65 minutes giving a minimum of 60 minutes at this temperature. Sbrinz is cooked at similarly high temperatures, *e.g.* 55-57^{o}C but held for longer initially (40-50 minutes). Bachmann and Spahr (1995) demonstrated a 3.5 log reduction in *E. coli* occurred when curd was heated at 53ºC for 45 minutes during the manufacture of Swiss Emmentaler cheese. The D-value of *E. coli* at 55°C is 5.5 minutes in skim milk and 6.6 minutes in whole milk (ICMSF, 1996). Using these values *E. coli* numbers would be reduced by approximately 6 logs during this processing step.

During the first 24 hour of cheese manufacture, any *E. coli* surviving in the milk will be concentrated in the curd through syneresis. If these organisms are not destroyed during the curd cooking, there is the possibility that they may multiply during subsequent salting and ripening phases, until the combination of low pH and water activity inhibit growth.

Table 3 describes the impact of cheesemaking on *E. coli* numbers in selected cheese types. The pH of these cheeses at salting ranges from 4.95 - 5.2 and the NaCl concentration of the final cheese is 1.3 - 1.6g/100g with the exception of Pecorino Romano that has a pH of 5.85 and a salt concentration of 5g/100g.

Table 3: Increase in *E. coli* numbers during cheesemaking and early ripening

Cheese	Strain	Stage (after)	Time from start (days)	pH (24 hours)	Log increase	Reference
Colby	Non-O157	Pressing	1	4.91 - 5.34	0.2 - 4.0	(Kornacki and Marth, 1982)
Colby	O157	Salting	<1		1.3	(Hudson *et al*., 1997)
Cheddar	3 strains O157	Pressing	1		0.7 - 1.4	(Reitsma and Henning, 1996)
Brick*	Non-O157	Brining	2		2.2 - 2.5	(Frank *et al*., 1978)
Tilsiter**	NCTC 9001	Brining	3		~1.5	(Bachmann and Spahr, 1995)
Romano***	O157	Brining	4		1.7	(Hudson *et al*., 1997)

* Brick: after 24 hours in 22% brine ** Tilsiter: after 24 hours in 20% brine and 1 day ripening at 11 - 13°C
*** Romano: after 65 hours in 22% brine

Hudson *et al*. (1997) reported an increase of 1.7 logs during curd formation of Romano cheese after 65 hours in 22% brine (Table 3). For hard cheeses the amount of growth of *E. coli* during the initial stages of ripening is likely to be less than 2 logs.

During ripening and storage, the numbers of *E. coli* present in the cheese will decrease. The rate of decrease will be primarily dependent on the storage temperature, although further reductions in pH and water activity will contribute to the rate of inactivation (Table 4). Not surprisingly, the results are variable, reflecting the heterogeneous nature of these cheeses and the strains of *E. coli* being evaluated.

Table 4: Decrease in *E. coli* numbers during late ripening and maturation of cheese

Cheese	*E. coli* strain	% salt in H_2O	pH	Ageing Conditions	Log decrease	Reference
Colby*	ETEC-a	3.7 - 4.9	4.9 - 5.3	6.5 wk/10°C	>3	(Kornacki and Marth, 1982)
Colby*	ETEC-b	3.9 - 4.0	5 - 5.6	11 wk/10°C	1.5-4	(Kornacki and Marth, 1982)
Colby*	EIEC-a	5.4 - 5.9	5.3 - 5.5	3.5 wk/10°C	>5	(Kornacki and Marth, 1982)
Colby**	O157 EHEC		4.6	4 wk/13°C	4	(Hudson *et al*., 1997)
Cheddar*	3 strains O157	3.15	5 - 5.2	22.5 wk/6 - 7°C	2.8-5.8	(Reitsma and Henning, 1996)
Cheddar*	3 strains O157	3.34	5 - 5.2	18.5 wk/ 6 - 7°C	~2.1	(Reitsma and Henning, 1996)
Brick***	ETEC-b		5.1 - 5.3	2 wk at 15.5°C + 5 wk at 7°C	0.64 – 2.4	(Frank *et al*., 1978)
Tilsiter†	NCTC 9001	3.13	5.2 - 5.4	30 d/11 - 13°C	6.5	(Bachmann and Spahr, 1995)
Romano††	O157 EHEC	-	5.2 - 5.7	2 d at10°C + 30d at 13°C	>4.5	(Hudson *et al*., 1997)

* Time is from start of ageing-maturation
** Time is from salting
*** Brick: after 24 h in 22% brine
† Tilsiter: after 24 h in 20% brine and 1 day ripening at 11 - 13°C
†† Romano: after 65 hours in 22% brine

Extra hard cheeses are matured for a minimum of three months and up to 24 months at temperatures as high as 15 - 22°C. While there is considerable variability in survival, reductions of greater than 4.5 logs have been observed during the maturation of Romano cheese for 30 days at 11 - 13°C (Table 4) giving an overall net effect for the cheesemaking process of a 3 log reduction. It is likely that maturation for longer periods will result in further reductions in *E. coli* numbers.

Raw milk extra hard cheese manufactured with a curd cooking temperature of >55°C for 30 minutes and matured for a minimum of 3 months will achieve greater than a 5 log reduction in *E. coli* numbers. Where extra hard cheeses receive a lower cooking temperature, they should be where possible manufactured with pasteurised milk or be ripened for a minimum of 6 months.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
Parmigiano Reggiano, Grana Padano and Sbrinz	Some initial growth. Curd cooking (55 - 56^{0}C 15 - 20 min and 55 - 57^{o}C 40 - 50 min) results in death of *E. coli.* (5 log)	Long maturation period at high temperatures likely to result in further significant death (5 log)	Eliminates
Pecorino Romano, Asiago and Montasio	Some initial growth (1- 2 log). Limited destruction during curd cooking. *E. coli* may survive.	Long maturation period results in reduction/death (> 4.5 logs)	Eliminates

3.1.3 *Salmonella* spp.

Most *Salmonella* spp. have similar thermal resistance to *E. coli*, with the D-value for most *Salmonellae* in milk of 3.6 - 5.7 seconds at 62.8°C. Some species such as *Salmonella Senftenberg* are unusually heat resistant, although these species are rare, especially in milk. The D-value for *S. Senftenberg* is 34 seconds at 65.6°C (ICMSF, 1996).

Where extra hard cheese is made from pasteurised milk *e.g.* Asiago and Montasio, *Salmonellae* will be inactivated during the heat treatment of the milk. Similarly, most *Salmonellae* will be destroyed if milk is thermised at 65-68°C for more than 10 seconds *e.g.* Pecorino Romano cheese.

While concentration may occur during syneresis, *Salmonella* spp. numbers in extra hard cheeses will decrease at high curd cooking temperatures. The D-value for *S.* Typhimurium at 51.4 and 55.2ºC in laboratory media containing 10% milk solids is 49.0 minutes and 4.7 minutes, respectively (ICMSF, 1996). Where curd is cooked at >55°C for a minimum of 30 minutes *i.e.* Parmigiano Reggiano, Grana Padano and Sbrinz, a >5 log reduction in *Salmonella* will be achieved. At lower curd cooking temperatures as found in some Romano, Asiago and Montasio cheeses (45 - 48ºC), little if any inactivation may occur. Bachmann and Spahr (1995) demonstrated a reduction of ~2 logs in *Salmonella* spp. after cooking at 53°C for 45 minutes and an increase of ~1 log after cooking at 42°C for 15 minutes. As the cheeses are ripened and matured the numbers of viable *Salmonellae* will decline (Table 5).

Table 5: Decrease in *Salmonellae* during ageing and maturation of cheese

Cheese	% Salt	pH	Ageing Conditions*	Log decrease	Reference
Cheddar		5.4 - 5.65	26 wk at 4.5°C	2.5	(Hargrove *et al*., 1969)
		5.2 - 5.3	26 wk at 4.5°C	5.3	
		5 - 5.05	13 wk at 4.5°C	5	
		5.2 - 5.4	13 wk at 10°C	4	
Cheddar			14-16 wk at 7.5°C	4	(Goepfert *et al*., 1968)
			10-12 wk at 13°C	4	
Cheddar	2.1 - 2.3	5.2	20 wk at 7°C	4.8 - 5.2	(Mehta and Tatini, 1994)
Samsoe		5.2	5-6 wk at 16-20°C + ca 3 wk at 10-12°C	4	(Goepfert *et al*., 1968)
Montasio		5.4 - 5.6	12-13 wk at 12°C	~4.5	(Stecchini *et al*., 1991)
Manchego	2.5 - 3	4.9 - 5.0	8 wk at 10°C	~7	(Medina *et al*., 1982)
Manchego***	2.5 - 3	4.9 - 5.0	6 wk at 10°C	4.6 - ~ 6.5	
Tilsiter***	1.23	5.2 - 5.4	4 wk at 11-13°C	6.3	(Bachmann and Spahr, 1995)

* Time and extent of decrease in *Salmonellae* in Cheddar is from 1 day after production; in the semi-hard cheeses, Montasio from day 3 (after brine-salting) and Tilsiter from day 3 (after brine-salting and 1 day ripening); and for Manchego, from day 2 (after brine-salting)

** Cheddar used by Mehta and Tatini (1994) had an a_W of 0.95-0.97

*** Internal salt content of Manchego is after 60 days and for Tilsiter after 90 days

High maturation temperatures and longer storage times will result in greater reductions in viable numbers of *Salmonella*. The maturation of a semi-hard cheese (pH 5.2-5.8, 39% moisture, 1.2% w/w salt) for 30 days at ~12°C results in a ~5 log reduction in *Salmonellae* (Bachmann and Spahr, 1995). The rate of inactivation appears to be linear implying that storage for 60 days would result in a ~7 log reduction.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
Parmigiano Reggiano, Grana Padano and Sbrinz	Some initial growth. Curd cooking (55-57^{0}C 1550 min) results in death of *Salmonella. (5 log)*	Long maturation period at high temperatures likely to result in further significant death (*5 log)*	Eliminates
Pecorino Romano, Asiago and Montasio	Some initial Growth (1- 2 log). Limited destruction during curd cooking. *Salmonella* may survive.	Long maturation period results in reduction/death (> 4.5 logs)	Eliminates

3.1.4 *Staphylococcus aureus*

S. aureus may be found in raw milk used to manufacture extra hard cheeses. *S. aureus* presents a particular problem because although the organism is sensitive to heat, it produces a heat-stable enterotoxin that survives the pasteurisation and cooking processes (ICMSF, 1996). For both raw milk and pasteurised milk cheeses, staphylococci could be present in the raw milk and management of herd health and control of hygiene during milk production are essential to avoid enterotoxin production in raw milk at levels that may cause illness.
S. aureus is inhibited by low pH and is a poor competitor; hence significant increases during cheesemaking generally reflect problems with the starter culture.

Exposure of raw milk to heat processes will significantly reduce the numbers of *S. aureus*. Using milk as a heating medium, the D-value for *S. aureus* at 65, 70 and 75°C have been reported to be 12, 6 and 1.2 seconds, respectively (ICMSF, 1996). Johnson *et al*. (1990b) summarised heat studies of milk used for cheesemaking and concluded that at 70°C a holding time of 16 - 18 seconds was required to inactive *S. aureus* (Johnson *et al*., 1990b). At 65°C, 63 seconds was required for inactivation. There is evidence that *S. aureus* may become injured and is not readily recovered after heat treatment (*e.g.* 63.9 - 65.6°C for 16 - 21 seconds), although recovery may occur during cheesemaking (Zottola *et al*., 1969). Based on these studies, the thermal treatment of milk at 73 - 75°C for 15 - 30 seconds will inactivate *S. aureus* (*i.e.* Asiago and Montasio cheeses). Treatment of milk at lower temperatures, *i.e.* at 65 - 68°C for Pecorino Romano cheese will inactivate the bacterium if held for at least 63 seconds.

Holding temperatures of 33 - 40°C for raw milk after the addition of the starter culture are favourable for the growth of *S. aureus*. However, as the milk is stored at this temperature for only a short period of time, and if the starter culture is of appropriate fecundity, the extent of growth would be minimal. Growth conditions are considered less favourable in raw milk as the lactoperoxidase system and the natural flora present inhibit staphylococcal growth (Bachmann and Spahr, 1995).

Cooking the curd at 55 - 56ºC for 15 - 20 minutes when making Parmigiano Reggiano and Grana Padano and 55 - 57^{o}C for 40 - 50 minutes for Sbrinz, will result in considerable destruction of *S. aureus*. The D-value in milk at 50 ºC is 10 minutes and at 55 ºC is 3 minutes (ICMSF, 1996). A 2 - 3 log reduction was noted after cooking at 53ºC for 45 minutes (Bachmann and Spahr, 1995). At lower cooking temperatures, between 42 - 48ºC, there is little effect on the numbers of *S. aureus*.

Initial increases of between 1 - 2 logs may occur during the manufacture of Swiss-type cheeses (with a cooking temperature up to 51ºC), due in part to the concentration of cells in the curd (Todd *et al*., 1981). Similar increases have been reported for semi-hard Swiss-type cheeses (Bachmann and Spahr, 1995). Although staphylococci can grow at temperatures as high as 48ºC, this temperature is near the maximum for growth and with a falling pH bacterial numbers are unlikely to reach those required for enterotoxin production. However if the pH does not fall rapidly, growth can occur. Todd *et al*. (1981) noted the work of Tatini *et al.* (1973) showing that *S. aureus* numbers can reach counts of 10^7 cfu/g in 24 hours where the pH only fell to between 5.5 - 5.6.

For Italian DOC hard cheeses the manufacturing protocols state that the pH should be <5.2 within 24 hours for the manufacture of Parmigiano Reggiano, Grana Padano and Pecorino Romano. Rapid pH fall is essential for preventing significant growth of *S. aureus*.

Toxin production appears to be more closely associated with growth in the milk prior to cheesemaking rather than as a result of temperatures allowing growth during curd formation (Todd *et al*., 1981). Temperature control of the raw milk used in cheesemaking is essential to ensure that *S. aureus* numbers remain below 10^6 cfu/ml, even if the milk is heat treated prior to use. Pre-formed *S. aureus* enterotoxin will survive curd heating protocols such as in traditional Canestrato Pugliese cheese (Pasta filata type) where the curd is exposed to hot whey at 80ºC for 30 seconds (Albenzio *et al*., 2001). Because some growth of *S. aureus* can occur during manufacture, numbers in the milk should be less than 10^3 cfu/ml at the start of manufacture.

Failure of starter cultures to rapidly lower the pH or temperature abuse of the raw milk would be required to allow *S. aureus* to reach the levels required for enterotoxin production. After fermentation counts may increase during salting. Todd *et al.* (1981) noted published work showing *S. aureus* numbers increasing to ~10^8 after 2 weeks followed by a gradual decrease in numbers until a 2 log total reduction (4 log maximum reduction) was achieved at 15 weeks. Bachmann and Spahr (1995) failed to find *S. aureus* in Swiss-type hard cheese immediately after cooking the curd (5 log reduction), while in semi-hard cheese a 5 log reduction was observed after maturation for >30 days.

S. aureus should be inactivated in hard cheeses manufactured with a curd cooking temperature of >55ºC (30 minutes) and matured for a minimum of 3 months. The presence of toxin appears to be determined by the number of organisms present in the milk used for cheesemaking. Some growth (1-2 logs) of *S. aureus* would be expected in cheeses receiving a lower cooking temperature, therefore *S. aureus* numbers in milk used in the manufacture of these cheeses should be less than 10^3 cfu/ml. Storage of these cheeses for 3-months should ensure that they are free of viable *S. aureus*.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
All extra hard raw milk cheeses	Initial growth. Rapid acidification will limit growth. Curd cooking in Parmigiano Reggiano, Grana Padano and Sbrinz decrease numbers.	Studies indicate limited survival after 1 day in hard cheese and not detectable after 90 days in semi-hard cheeses.	99% reduction

3.1.5 *Listeria monocytogenes*

Listeria spp. numbers decrease considerably during thermal treatment of milk. D-values in whole milk heated to 52.2ºC range between 24 - 37 minutes, while at 57.8 ºC the D-value is 4.4 - 5.2 minutes (ICMSF, 1996). The D-value in milk at 63.3ºC is 33.3 seconds and at 68.9ºC is 7.0 - 7.2 seconds (Johnson *et al*., 1990b). Thermisation for sufficient time and pasteurisation will inactivate *Listeria* spp. present in the raw milk.

During the production of parmesan cheese *Listeria* spp. numbers increased during the initial heating stages before decreasing by approximately 0.45 logs during cooking at 51ºC for 45 minutes (Yousef and Marth, 1990).

Cooking at a temperature of 53ºC for 45 minutes (pH 5.2 - 5.4) resulted in a >4.6 log reduction in *Listeria* spp. (Bachmann and Spahr, 1995; Spahr and Url, 1994), cooking at 56ºC for 25 minutes gave a similar reduction (Spahr and Url, 1994). Heating whole milk at 55ºC for 40 minutes should result in only a ~2 log decrease in *Listeria* spp. (from D-values in whole milk); this is lower than expected from the results in experiments carried out on curd.

Cooking the curd at lower temperatures *e.g.* 42 - 48ºC will have limited if any effect on the viability of *L. monocytogenes*. In Swiss-type semi-hard cheese made from experimentally inoculated milk, after cooking the curd at 42ºC for 15 minutes a slight increase in *L. monocytogenes* was observed, probably due to concentration during syneresis.

Survival of *Listeria* spp. during the manufacture of cheese is highly variable between products and within the same product. *Listeria* spp. have been shown to survive and even grow on the outside surface of cheese during maturation. Survival is dependent on the pH of the cheese and at levels of ~pH 5.5 no growth should occur on the outer surface of hard cheeses (Bachmann and Spahr, 1995). Genigeorgis *et al*. (1991) examined the survival of *Listeria* spp. on the surface of a variety of cheeses. Non-soft cheeses made with the use of starter cultures and at pH values of ≤5.5 did not support the growth of *L. monocytogenes* at temperatures ranging between 4 - 30ºC. Contamination of the outside surface of cheese with *Listeria* spp. should be similar for cheeses manufactured from raw or heat-treated milk (Genigeorgis *et al*., 1991).

The combination of cooking at 51ºC, low pH and high storage temperature (pH 5.1 and 12.8ºC) used in the manufacture of Parmesan cheese reduced the most persistent strain of *Listeria* by >4.5 logs after 120 days (Yousef and Marth, 1990). The pH and water activity of ripened Sbrinz cheese (5.5 - 5.7 and 0.94, respectively) would suggest that it is unlikely that growth of *L. monocytogenes* would occur.

Cooking of the curd to temperatures of >55ºC followed by maturation at >15ºC for more than 3 months will result in reductions in *Listeria* spp. of more than 5 logs. Cooking at lower temperatures may result in a slight increase in *Listeria* spp. during the early stages of production, followed by a rapid decrease in numbers during storage, reaching >5 logs after approximately 6 months.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
Parmigiano Reggiano, Grana Padano and Sbrinz	Curd cooking reduce numbers of *L. monocytogenes* (>4.6 log)	Long maturation period at high temperatures likely to result in further significant death (5 log)	Eliminates
Pecorino Romano Asiago and Montasio	Some growth during coagulation, curd cooking and pressing	pH 5.1 and high storage temperature result in decreases of approximately >4.5 log	Eliminates

3.2. Consumption of extra hard cheeses in Australia

Data from the 1995 Australian National Nutrition Survey[35] (NNS) gives an indication of the percentage of the population who consume various types of cheese and the amount they consume.

Extra hard cheese is not a major food item consumed by Australians and is normally only consumed in small volumes. Consumption data from the NNS shows that only 2% of those surveyed consumed extra hard cheese, with an average amount consumed of 8 g/day

[35] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24-hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24-hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

(Table 6). Table 6 shows data from the NNS on the Australian average daily consumption of very hard cheese by gender and age. Extra hard cheese was consumed by all age groups.

It cannot be assumed that this same proportion of the population would also consume raw milk cheese. However, it is likely that those who will consume raw milk cheese, will not increase their cheese consumption, rather they will substitute consumption of pasteurised cheese with raw milk cheese.

Table 6: Australian average daily consumption of very hard cheese by gender and age (Australian Government Department of Health and Family Services, 1997)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)
Male	2 -3	170	2	(1.17)	4.25
Male	4 - 7	416	8	(1.92)	4.55
Male	8 - 11	385	7	(1.82)	2.51
Male	12 - 15	349	2	(0.58)	5.95
Male	16 - 18	215	6	(2.79)	11.50
Male	19 - 24	485	11	(2.27)	32.26
Male	25 - 44	2140	57	(2.66)	10.67
Male	45 - 64	1554	32	(2.06)	13.93
Male	65+	902	13	(1.44)	9.72
Female	2 - 3	213	1	(0.47)	1.70
Female	4 - 7	383	7	(1.82)	8.30
Female	8 - 11	354	9	(2.54)	5.01
Female	12 - 15	304	9	(2.96)	1.78
Female	16 - 18	218	8	(3.67)	6.80
Female	19 - 24	575	11	(1.91)	7.38
Female	25 - 44	2385	79	(3.31)	7.96
Female	45 - 64	1752	41	(2.34)	9.86
Female	65+	1058	14	(1.32)	8.42

4 Risk characterisation

In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles (Appendix 1).

The qualitative framework considers the characterisation of identified hazards (hazard identification and characterisation combined) and an assessment of the likely exposure to these hazards (exposure assessment) which when combined provides a characterisation of the risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of disease. The exposure module characterises exposure to the hazard based on the likely level of the hazard in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of the hazard on a "per serve" basis[36]. Essentially the matrix categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

[36] "per serve" is defined as the amount of product consumed per eating occasion.

The model was employed to characterise the risk from high temperature cook and low temperature cook extra hard cheese.

4.1 High temperature curd cook

Risk categories for hazards in extra hard raw milk cheeses were assigned as follows:

Pathogen	Infective dose	Consequence of exposure	Severity of hazard
C. jejuni	100 - 1,000	Moderate/Serious#	Very Low/Low#
E. coli (EHEC)	<10	Serious	High
Salmonella spp.	10 - 100	Moderate/Serious	Low/Moderate#
S. aureus	>1,000	Mild	Negligible
L. monocytogenes	>1,000/10 - 100#	Moderate/Severe#	Negligible/Moderate#

susceptible populations

Exposure categories for extra hard raw milk cheeses exposed to a high temperature cook were assigned as follows:

Pathogen	Raw product contamination	Effect of processing	Exposure
C. jejuni	Infrequent (1%)	Eliminates	Negligible
E. coli (EHEC)	Infrequent (1%)	Eliminates	Negligible
Salmonella spp.	Infrequent (1%)	Eliminates	Negligible
S. aureus	Sometimes (10%)	Eliminates	Negligible
L. monocytogenes	Infrequent (1%)	Eliminates	Negligible

Risk characterisation for raw milk extra hard (high temperature curd cook) cheese:

Pathogen	Hazard charcterisation	Exposure assessment	Risk Characterisation
C. jejuni	Very low/Low#	Negligible	Negligible
E. coli (EHEC)	High	Negligible	Low
Salmonella spp.	Low/Moderate#	Negligible	Negligible/Very Low#
S. aureus	Negligible	Negligible	Negligible
L. monocytogenes	Negligible/Moderate#	Negligible	Negligible/Very Low#

susceptible populations

4.2 Low temperature curd cook

Exposure categories for raw milk extra hard cheeses made from milk subjected to a low temperature cook were assigned as follows:

Pathogen	Raw product contamination	Effect of processing	Exposure
C. jejuni	Infrequent (1%)	Eliminates	Negligible
E. coli (EHEC)	Infrequent (1%)	Eliminates	Negligible
Salmonella spp.	Infrequent (1%)	Eliminates	Negligible
S. aureus	Sometimes (10%)	99% Reduction	Very Low
L. monocytogenes	Infrequent (1%)	Eliminates	Negligible

Risk characterisation for raw milk extra hard (low temperature curd cook) cheese:

Pathogen	Hazard charcterisation	Exposure assessment	Risk Characterisation	
C. jejuni	Very Low/Low#	Negligible	Negligible	
E. coli (EHEC)	High	Negligible	Low	
Salmonella spp.	Low/Moderate#	Negligible	Negligible/Very Low#	
S. aureus	Negligible	Very low	Negligible	
L. monocytogenes	Negligible/Moderate#	Negligible	Negligible/Very Low#	

susceptible populations

5 Conclusions

Extra hard cheeses represent a product which has a low likelihood of contamination with pathogenic microorganisms such as *C. jejuni/coli*, enterohaemorrhagic *E. coli*, *Salmonella* spp., *S. aureus* and *L. monocytogenes*. While these organisms may be present in the raw milk, and in some situations increase in numbers by 1-2 logs during the initial phase of cheesemaking (warming and holding the raw milk when the starter culture is first added), these organisms will be destroyed during the cooking of the curd and/or during the prolonged ripening of this class of cheeses.

Cheeses such as Parmigiano Reggiano, Grana Padano and Sbrinz are exposed to a high temperature curd cook *i.e.* 55 - 57°C resulting in significant destruction of these pathogens, typically greater than 5 log reductions. Where lower cooking temperatures are used there is less destruction. The use of pasteurised or thermised milk for the manufacture of Pecorino Romano, Asiago and Montasio cheeses adds additional safety.

Inactivation of pathogens continues throughout ripening (providing the pH is 5.5 or less) and reductions of >5 logs occur when ripening extends beyond 3 months regardless of the curd cooking temperature.

The presence of *S. aureus* in raw milk is a risk factor, and temperature control is necessary for raw milk, whether it is destined for raw milk or pasteurised milk cheeses. The growth of *S. aureus* in the raw milk can result in enterotoxins which may then persist in the final cheese. The cheesemaking process will not inactivate enterotoxin and it can persist in the cheese for long periods of time. *S. aureus* will not grow in hard cheeses receiving a cook of >55°C, therefore enterotoxins can only originate from the milk.

All extra hard cheeses made according to the process criteria identified during this assessment will achieve a 5 log reduction in the numbers of the pathogens specified *e.g. C. jejunicoli*, *S. aureus*, *L. monocytogenes*, pathogenic *E. coli*, and *Salmonella* spp. The process of manufacturing extra hard cheese makes it unlikely pathogens will survive or proliferate. This is confirmed by a review of foodborne illness associated with extra hard raw milk cheeses that found this class of cheese has not been implicated in any outbreaks of foodborne illness.

The process of manufacturing extra hard raw milk cheese has been assessed to affect the selected pathogens as follows:

Pathogen	***Risk associated with raw milk extra hard cheese***
Campylobacter spp.	*Campylobacter* spp. are unlikely to survive processing and maturation and are a **negligible** risk.
E. coli (EHEC)	**Low** risk as the organism doesn't survive the curd cooking process in the high curd cook cheeses or cheese maturation.
Salmonella spp.	**Negligible** risk (general population) and **very low** risk (susceptible population) as the organism doesn't survive the curd cooking process in the high curd cook cheeses or cheese maturation.
S. aureus	Risk from *S. aureus* is considered **negligible**. Conditional on good control over animal health and raw milk handling to prevent growth of the organism to numbers where toxin production is possible.
L. monocytogenes	**Negligible** risk (general population) and **very low** risk (susceptible population) as the organism doesn't survive the curd cooking process in the high curd cook cheeses or cheese maturation.

There is no difference in the public health and safety risk from any of the selected pathogens in extra hard raw milk cheeses made from cow, goat or sheep milk.

Challenge studies on the fate of specific pathogens in extra hard raw milk cheeses support their designation as being safe. Pellegrino and Resmini (2001) noted that curd cooking temperatures, low water activity and extended ripening times of Grana Padano and Parmigiano Reggiano cheeses was inhibitory to bacterial pathogens (Pellegrino and Resmini, 2001). Hudson *et al.* (1997) examined the fate of *E. coli* O157 in Romano cheese and found a >4.5 log reduction (Hudson *et al*., 1997). Pecorari *et al.* (2001) examined the fate of *E. coli*, *S. typhimurium*, *S. aureus* and *L. monocytogenes* during production and ripening and found that none of the inoculated pathogens were detected 24 hours after cheesemaking (Pecorari *et al*., 2001). Yousef and Marth (1990) also found rapid decline of *L. monocytogenes* to undetectable levels within 14 –122 days (Yousef and Marth, 1990).

The microbiological safety of extra hard cheeses is dependent upon the microbiological quality of the raw milk, rapid acidification (to prevent growth of *S. aureus* to levels that may produce enterotoxin), curd cooking, and maturation.

The findings of the raw milk extra hard cheeses assessed may be applied to the entire extra hard cheese category as they generally have similar physicochemical characteristics and manufacturing protocols *e.g.* curd cooking and long ripening times.

APPENDIX 9: Risk assessment – Swiss-type raw milk cheeses

1 Introduction

Swiss-type cheeses are classified as either hard or semi-hard and are characterised by propionic acid fermentation leading to the formation of eyes or mechanical openings resulting from the incomplete fusion of curd pieces and the production ofCO_2. Swiss-type cheeses may be made using raw or thermised milk[37] and includes varieties such as Emmentaler, Gruyère, Appenzeller, Tilsiter, Vacherin Fribourgeois and Tête de Moine.

Emmentaler is a hard yellow cheese made from cow milk, with a mild, nutty taste distinguished by large holes that are formed by pockets of gas. Gruyere differs from Emmentaler by being produced in smaller wheels and having a somewhat stronger flavour and fewer eyes. In addition, Gruyere is characterised by the development of a surface flora (similar to that which develops on smear-ripened varieties). Appenzeller is a small cheese with a soft texture. Wine or cider is sometimes applied to the wheels during curing to flavour and preserve the cheese whilst promoting rind formation. Tilsiter, Vacherin Fribourgeois and Tête de Moine are medium-firm textured Swiss-type cheeses with irregular holes or cracks.

This risk assessment qualitatively examined the fate of the *Campylobacter jejuni*, *Escherichia coli* (EHEC), *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* during the manufacture of Emmentaler, Gruyère, Appenzeller, Tilsiter, Vacherin Fribourgeois and Tête de Moine raw milk Swiss-type cheeses.

A qualitative framework developed by Food Science Australia was subsequently used to rate the risk to public health and safety from the consumption of raw milk Swiss-type cheese made from cow, goat or sheep milk, containing these microbiological hazards.

The information contained in this risk assessment is based on work previously undertaken by FSANZ during the evaluation of Application A357 – Swiss Raw Milk Cheeses[38]. At this time, the then Australia New Zealand Food Authority (the precursor to FSANZ) received an application from the Swiss Federal Veterinary Office requesting a variation to Standard H9 - Cheese and Cheese Products, in the *Australia New Zealand Food Standards Code.* The request sought permission to import the Swiss-type Emmentaler, Gruyère, Appenzeller, Tilsiter, Vacherin Fribourgeois, Tête de Moine cheeses and the extra hard Sbrinz cheese. As part of this Application, a risk assessment of the manufacturing protocols set out in the Swiss Federal Government Ordinances was undertaken.

Emmentaler, Gruyère and Sbrinz were ultimately permitted, while approval for Appenzeller, Tilsiter and Vacherin Fribourgeois were given on the basis that they were made from thermised milk. Tête de Moine is made exclusively from raw milk, and was not assessed as safe.

The specific manufacturing processes assessed for Appenzeller, Tilsiter and Vacherin Fribourgeois cheeses included thermal treatment of the milk (thermisation). While the significant effect that thermisation would have on the pathogens evaluated was noted, the risk assessment analysed the effect of the curd cooking processes and the effects of ripening and

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[37] Raw milk heat treated to a minimum temperature of 62°C for a period of not less than 15 seconds.

[38] http://www.foodstandards.gov.au/_srcfiles/A357%20FAR.pdf

storage on bacterial reduction. This allowed for an evaluation of the production processes on pathogen survival for these cheese types if the raw milk used was not subject to thermisation.

2 Hazard identification and hazard characterisation

In evaluating the safety of raw milk Swiss-type cheeses, the following pathogens were considered: *C. jejuni/coli*, pathogenic *E. coli*, *L. monocytogenes*, *Salmonella* spp. and *S. aureus*.

A detailed characterisation of these hazards is attached at Appendix 14.

3 Exposure assessment

The main steps in the manufacture of Swiss-type cheese are outlined in Figure 1.

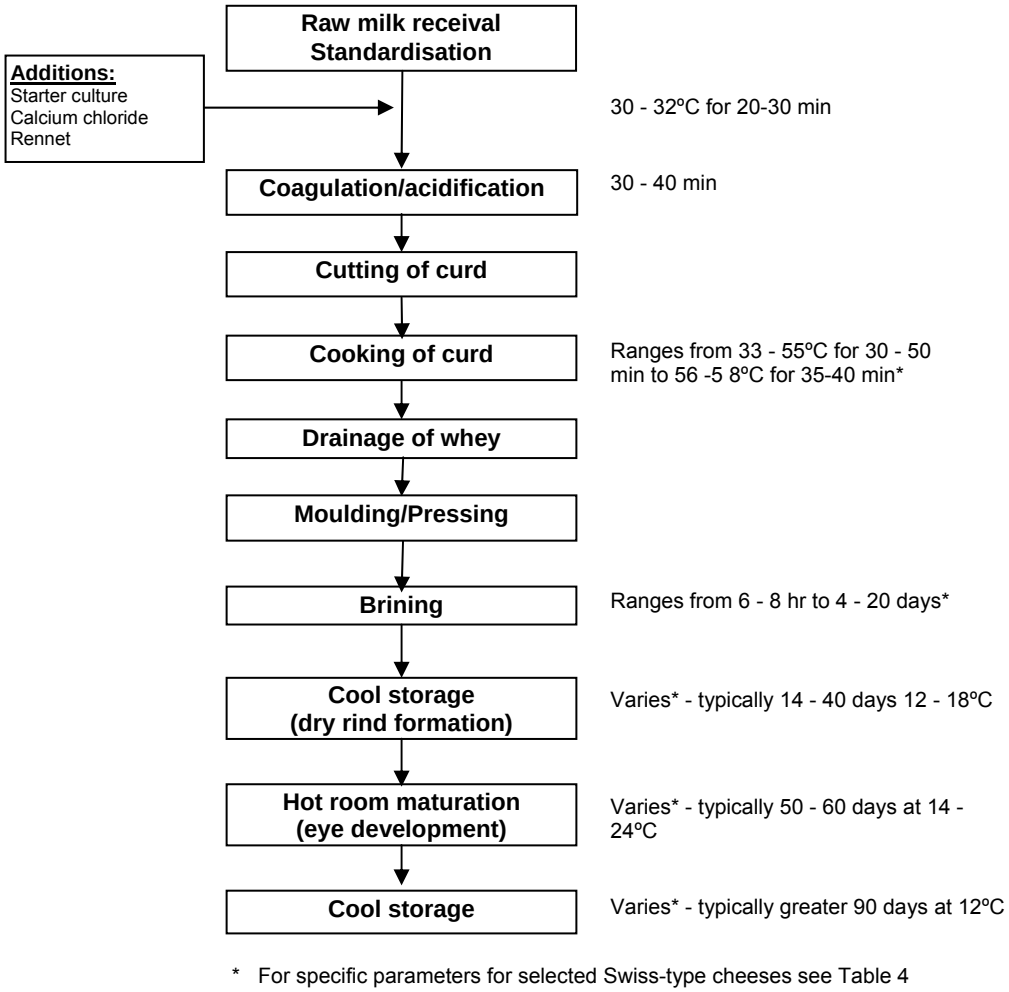

Figure 1: Overview of major steps in the manufacture of Swiss cheese

In Swiss cheese, carbon dioxide is produced by *Propionibacterium freudenreichii* spp. *shermanii* from lactate during the ripening phase which causes the formation of eyes. These proprionibacteria do not grow in the milk during cheesemaking, but grow in the cheese during maturation when the cheese is transferred to a warm ripening room (~20-22°C).

The actual steps in the preparation of Swiss-type cheeses vary significantly between the individual styles, as described in Table 1.

Table 1: Summary of processing steps for individual Swiss-type raw milk cheeses

Processing step	Appenzeller	Emmentaler	Gruyère	Tête de Moine	Tilsiter	Vacherin Fribourgeois
Raw milk heat treatment	Sometimes thermised	nil	nil	nil	Sometimes thermised	Sometimes thermised
Acidification/ whey starter added	Milk warmed to 30ºC for 20 min	Milk warmed to 31 - 32ºC for 30 - 40 min	Milk warmed to 30 - 32ºC pH 6.4	Milk warmed to 30 - 32ºC for 20 - 30 min	Milk warmed to 30 - 32ºC 20 - 30 min	Milk warmed to 32ºC for 30 min
Coagulation/ rennet addition	30 min	40 min	30 - 40 min	30 min	30 min	30 min
Curd cutting						
Curd cooking	42 - 46ºC for 30 - 50 min	52 - 53ºC for 30 - 40 min	56 - 58ºC for 35 - 40 min	44 - 46ºC for 15 - 30 min	42 - 46ºC for 15 - 30 min	33 - 35ºC for 30 - 50 min
Curd resting, hooping and pressing		Mechanical pressing	Mechanical pressing	Mechanical pressing	Mechanical pressing	
Salting	Brining 1 - 3 days at 10 - 14ºC	Brining 1 - 3 days at 12 - 14ºC	Brining 1 - 2 days at14ºC	Brining 1 day	Brining 1 - 2 days at 10 - 14ºC	Brining 6 - 8 hr at 10 - 12ºC
Ripening	16ºC for 6 weeks Regularly turned and surface treated Further aged 90 days	14 days at 12 - 14ºC second ageing period 50 - 60 days at 20 - 24ºC 20 - 50 days at 12ºC Sold at 120 days pH <5.7	15 - 16ºC for 40 days Another 60 days 14 - 15ºC Regularly turned and surface treated Ripened up to 120 days	12 - 16ºC for 28 - 49 days Regularly turned and surface treated Stored at 12°C Warehouse min age 120 days	16ºC for 4 weeks Sold at 90 days	16ºC for 6 weeks Regularly turned and surface treated Minimum age 90 days

The variations in manufacturing steps described in Table 1 result in different intrinsic properties for each cheese type, and influence the extent to which pathogenic microorganisms are eliminated or controlled. The resulting Swiss raw milk cheeses have varying physicochemical characteristics (Tables 2 and 3).

Table 2: Characteristics for individual Swiss-type raw milk cheeses

Property	Appenzeller	Emmentaler	Gruyère	Tête de Moine	Tilsiter	Vacherin Fribourgeois
pH	5.9	5.6	5.7	5.8	5.8	5.2-5.4
Water activity	0.96	0.97	0.95	0.93	0.96	>0.96
Moisture (%)	37-39%	37.2 (35-37%)	34.5(36%)	38-40%	39-41*	42-44%*
NaCl (%)	4 (s/m)	1.2 (w/w)	1.1 (w/w) 3.3 (s/m)		2.63 (w/w) 4.4 (s/m)	

w/w weight for weight

s/m salt in moisture (water phase)

Table 3: pH profile for individual Swiss-type raw milk cheeses

Time	Appenzeller	Emmentaler	Gruyere	Tête de Moine	Tilsiter	Vacherin Fribourgeois
0 hrs	6.4	6.35	6.4	6.4	6.4	-
2 hrs	5.9	5.85	6.15	6.0	6.0	-
4 hrs	5.45	-	5.8	5.5	5.5	-
5 hrs	-	5.5	-		-	-
8 hrs	5.2	-	5.45	5.35	5.35	-
10 hr	-	5.25	-	-	-	-
24 hr	5.2	5.2	5.2	5.2	5.2	5.05 - 5.15
10 d	5.3	5.44	5.3	5.25	5.25	-
20 d	-	-	-	-	-	-
30 d	5.37	5.52	5.55	5.4	5.4	-
60 d	5.6	5.63	5.63	5.6	5.6	-
90 d	-	5.68	5.7	-	-	5.2 - 5.4
120 d	-	-	-	-	-	-
130 d	5.9	-	-	5.85	5.85	-
150 d	-	5.7	-	-	-	-

- No data available

3.1 Survival of pathogens during the manufacture of specific Swiss-type cheeses

3.1.1 *Campylobacter jejuni/coli*

Campylobacter spp. are unlikely to grow in milk or cheese as the conditions for growth require temperatures between 32 - 45°C and optimum growth needs an elevated atmosphere of CO_2 and reduced oxygen tensions (ICMSF, 1996). Immediately before and after rennet addition in the manufacture of these Swiss-type cheeses, milk is held at 30 - 32°C but *Campylobacter* spp. are unlikely to grow. Similarly, milk holding temperatures for some cheeses (Appenzeller, Tilsiter and Vacherin Fribourgeois) during curd formation are close to the optimum for *Campylobacter* spp. growth, but the short holding time (less than 1 hour), the falling pH and the absence of microaerophilic conditions make it unlikely that significant growth will occur. Heating during curd production of Emmentaler and Gruyere cheeses will be lethal to *Campylobacter* cells as D-values for this microorganism in milk are about 4 - 5 minutes at 50°C and 0.7 - 1 minute at 55°C (ICMSF, 1996)

Campylobacter spp. do not survive well in mildly acidic environments, in the presence of 2% or more salt, or at water activities of <0.987 (ICMSF, 1996). Conditions in Swiss-type cheeses after brining and during curing and storage will be lethal for the organism and it would not be expected to survive.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
All Swiss-type cheeses	Initial growth unlikely due to falling pH. Lethal effect of curd cooking in Emmentaler and Gruyere.	Do not survive mildly acidic conditions or water activity <0.987.	Eliminates

3.1.2 Pathogenic *E. coli*

The ability of pathogenic *E. coli* to survive and or proliferate in Swiss-type cheese is influenced by manufacturing conditions including heat treatment, fermentation and ripening.

Gruyere, Emmentaler and Tête de Moine are made from raw milk and do not undergo any initial heat treatment of the raw milk. Thermised milk is usually used in the manufacture of Appenzeller, Tilsiter, and Vacherin Fribougeois. Thermisation conditions range from 57 - 68°C for 15 seconds and will have a variable effect on destroying *E. coli*. At the lower end of this temperature range, there will be little destruction (D-value: 1.3 - 3 minutes at 57°C; 45 - 47.4 seconds at 60°C; (ICMSF, 1996) while at 64 - 68°C, significant destruction of *E. coli* cells will occur (D-value at 64°C is 3 - 9.6 seconds).

E. coli present in milk used for cheesemaking become entrapped and concentrated in the curd and grow during fermentation (Frank *et al*., 1978; Hudson *et al*., 1997; Kornacki and Marth, 1982; Reitsma and Henning, 1996; Spahr and Url, 1994). Most growth occurs in the first 7 - 10 hours of cheese manufacture before inhibitory pH levels are attained. Some indication of the expected range of growth shortly after the start of manufacture is shown in Table 4. Growth slows with the addition of salt and will cease when the pH falls to about 5.2 (Frank and Marth, 1977).

Table 4: Increase in *E. coli* numbers in initial stages of cheese production

Cheese*	*E. coli* strain	Log increase	Stage	Time (days)	pH at 24 hr	% Salt in Water	Reference
Colby	ETEC-a	0.2 - 0.3	After press	1	4.91 - 4.99	3.7 - 4.9	(Kornacki and Marth, 1982)
Colby	ETEC-b	3 - 4	After press	1	5 - 5.25	3.9 - 4.0	(Kornacki and Marth, 1982)
Colby	EIEC-a	2 – 2.1	After press	1	5.26 - 5.34	5.4 - 5.9	(Kornacki and Marth, 1982)
Colby**	EHEC-0157	1.3	After salt	<1			(Hudson *et al*., 1997)
Cheddar	3 strains 0157	0.7- 1.4	After press	1		3.2 - 3.3	(Reitsma and Henning, 1996)
Romano***	EHEC-0157	1.7	After brine	4	5.2 - 5.7		(Hudson *et al*., 1997)
Brick†	ETEC-b	2.2	After brine	2	5.1 - 5.3		(Frank *et al*., 1978)
Brick†	EIEC-b	2.5	After brine	2	5.1 - 5.3		(Frank *et al*., 1978)
Brick†	EIEC-a	2.4	After Brine	2	5.2 - 5.3		(Frank *et al*., 1978)
Tilsiter	DSM30083	ca 1.5	After Brine	3	5.2 - 5.4		(Bachmann and Spahr, 1995)

* Time is from start of production
** Time and extent of decrease is from salting
*** Romano after 65 hr in 22% brine
† Brick after 24 hr in 22% brine
†† Tilsiter after 24 hr in 20% brine and 1 day ripening at 11-13°C

DSM 30083 non-pathogenic *E. coli*
ETEC Enterotoxogenic *E. coli*
EIEC Enteroinvasive *E. coli*
EHEC Enterohaemorrhagic *E. coli*

Heat treatment during curd formation also impacts on cell destruction. High curd cook temperatures *e.g.* Gruyere (55 - 57°C for one hour), significantly reduce *E. coli* numbers (D-value at 55°C = 5.5 - 8.5 minutes; (ICMSF, 1996), while lower curd cook temperatures *e.g.* Emmentaler (52 - 53°C for 30 - 40 min) have less effect. Experiments by Bachmann and Spahr (1995) indicate a 3.5 log reduction in *E. coli* numbers could be expected in Emmentaler cheese when the curd is heated at 53°C for 45 minutes.

Slow cooling of large pressed curd blocks (>20kg blocks of Gruyere and >70kg blocks of Emmentaler) prolongs time spent within the lethal temperature range and results in further die-off.

Temperatures used in setting (30 - 32°C) and subsequent curd heating for Appenzeller and Tilsiter (42 - 46°C); Tête de Moine (44 - 46°C); and Vacherin Fribougeois (33 - 35°C) could permit growth of *E. coli*. Entrapment and growth of *E. coli* is problematic in these Swiss-type cheeses since these curd-heating temperatures (33 - 46°C) are not lethal. *E. coli* numbers in the pressed curd are likely to be at least 10 - 100 times those initially present in the raw milk.

The number of *E. coli* present in cheese declines during ripening, after salting. The extent and rate of decline is quite variable and strain dependant. Death of *E. coli* in cheese is accelerated by a decrease in pH or water activity and by an increase in ageing temperature. A number of studies have examined the fate of *E. coli* strains in various hard-type cheeses as shown in Table 5.

Table 5: Effect of ageing and maturation on *E. coli* in cheese

Cheese*	*E. coli* strain	% salt in water	pH	Ageing conditions	Log decrease	Reference
Colby	ETEC-a	3.7 - 4.9	4.9 - 5.3	6.5 weeks at 10°C	>3	(Kornacki and Marth, 1982)
Colby	ETEC-b	3.9 - 4.0	5 - 5.6	11 weeks at 10°C	1.5-4	(Kornacki and Marth, 1982)
Colby	EIEC-a	5.4 - 5.9	5.3 - 5.5	3.5 weeks at 10°C	>5	(Kornacki and Marth, 1982)
Colby**	EHEC-O157		4.6	4 weeks at 13°C	4	(Hudson *et al*., 1997)
Cheddar	3 strains O157	3.15	5 - 5.2	22.5 weeks at 6-7°C	2.8 - 5.8	(Reitsma and Henning, 1996)
Cheddar	3 strains O157	3.34	5 - 5.2	18.5 weeks at 6-7°C	ca 2.1	(Reitsma and Henning, 1996)
Cheddar	5 strain O157:H7	3.34 - 4.66	5.28	26 weeks at 6-7°C	1-2	(Schlesser *et al*., 2006)
Brick	ETEC-b	After 24 hr in 22% brine	5.1 - 5.3	2 weeks at 15.5+ 5 weeks at 7°C	0.64 – 2.4	(Frank *et al*., 1978)
Brick	EIEC-a		5.2 - 5.3	2 weeks at 15.5 + 5 weeks at 7°C	1.46	(Frank *et al*., 1978)
Tilsiter	DSM30083	3.13 in rind (after 24 hr in 20% brine)	5.2 - 5.4	30 days at 11-13°C	6.5	(Bachmann and Spahr, 1995)
Romano	EHEC-O157	After 65hr in 22% brine	5.2 - 5.7	2 days at 10°C + 30 days at 13°C	>4.5	(Hudson *et al*., 1997)

* Time is from start of ageing-maturation
** Time and extent of decrease is from salting
ETEC Enterotoxogenic *E. coli*
EIEC Enteroinvasive *E. coli*
EHEC Enterohaemorrhagic *E. coli*
DSM 30083 non-pathogenic *E. coli*

Elevated ripening temperatures (16°C) and low pH (5.2 - 5.5) result in significant destruction of *E. coli* and the longer the ripening period, the greater the die-off. Protocols of Manufacture[39] which specify minimum shipping ages such as 120 days or 20 months encourage long ripening periods.

For Emmentaler cheese, the initial ripening conditions of 14 days at 12 -1 4°C would have less effect on *E. coli* than the second ripening period of 50 - 60 days at 20 - 24°C. At this more elevated temperature, *E. coli* would be metabolically more active and would succumb to the effect of the hurdles such as sub-optimal pH (< 5.7), salt concentration, and volatile fatty acid content (about 120 mmol/kg) (Steffen *et al*., 1993).

[39] Protocols of Manufacture as set out by the Federal Dairy Research Institute (Switzerland)

In Tilsiter-style cheese (3.1 - 4% salt in the water phase), a reduction of around 6.5 log units in numbers of one strain of *E. coli* has been reported during 30 days of ripening at 11 - 13°C (Bachmann and Spahr, 1995). This is considerably more death than observed for other cheeses (*e.g.* Cheddar and Colby) in which the pH, water activity and % salt are similar (Table 5). This may be due to the higher ripening temperature of 11 - 13°C for 30 days (Bachmann and Spahr, 1995). The Protocols of Manufacture for this cheese indicate this cheese is ripened at 16°C, which would be expected to result in greater die-off.

Appenzeller is similar in pH and water activity to Tilsiter. Ripened Appenzeller contains about 4% salt in the water phase, 74 mmol/kg of volatile fatty acids and a water activity of 0.96 (Steffen *et al*., 1993). Reduction in death rate due to increasing pH during ripening is offset by increasing amounts of volatile fatty acids which would be expected to accelerate death. In addition, the higher ripening temperature for Appenzeller cheese would result in a greater death rate of *E. coli* in comparison to Colby cheese which has similar pH and salt content. Log decreases for Colby range from 1.5 -
5 logs (Table 5), therefore a reduction in the number of *E. coli* may range from 2 - 5 logs during ripening of Appenzeller.

Maturation of Tête de Moine cheese occurs at 12 - 16°C for 28 - 40 days, followed by storage at 12°C for a minimum of 120 days before shipping. By day 60, the pH has risen from 5.2 to 5.6 with water activity after ripening at 0.93. The extent of death of *E. coli* during ageing and storage is unknown but may be greater than for Swiss Appenzeller and Tilsiter cheeses. Although the pH profile during production is similar, the lower water activity and longer storage time of Tête de Moine cheese would increase *E. coli* death. It is difficult to know the extent of this decrease as little information exists on the destruction of *E. coli* during the manufacture of this cheese.

Vacherin Fribougeois cheese maturation occurs at 14 - 16°C for 30 - 42 days with a minimum shipping age of 90 days. The pH rises from about 5.1 to 5.2 - 5.4 during ripening. Although the cheese wheel is about the same size as Appenzeller (6 - 7 kg), a shorter brining time (6 - 8 hr compared to 1 - 3 days), and greater water content (about 43% compared to 38%) would result in a higher water activity than that of Appenzeller or Tilsiter. It is difficult to compare the extent of *E. coli* death during ageing and storage between Vacherin Fribougeois, Appenzeller and Tilsiter cheeses. The lower pH profile would tend to give a faster death rate, but the higher water activity would tend to reduce it. There appears to be no information on the destruction of *E. coli* during the manufacture and ripening of Swiss Vacherin Fribougeois cheese.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
Gruyere	Some initial growth. Curd cooking results in death of *E. coli.*	pH 5.2-5.5 results in significant death	Eliminates
Emmentaler	Some initial growth. Destruction during curd cooking *(3.5 log)*	Likely significant death	Eliminates
Appenzeller, Tilsiter	Some initial growth. Temperatures permit growth until pH reaches 5.2 (100 fold)	Reduction/death of *E. coli* by 4-5 logs	Eliminates
Tête de Moine		pH profile similar to Appenzeller and Tilsiter cheeses, but lower water activity would increase death	Eliminates
Vacherin Fribougeois		pH would give faster death rate than Appenzeller and Tilsiter cheeses; but higher water activity may reduce it	Eliminates

3.1.3 *Salmonella* spp.

The survival and proliferation of *Salmonella* spp. in Swiss-type cheeses is influenced by manufacturing conditions including heat treatment, fermentation and ripening.

During the initial stages of cheesemaking, *Salmonellae,* will be concentrated in the curd, and are likely to grow and often reach about 100 times that in the original milk within the first 6 - 10 hours (Goepfert *et al*., 1968; Hargrove *et al*., 1969; Medina *et al*., 1982; Mehta and Tatini, 1994; Spahr and Url, 1994).

Where raw milk is subjected to thermisation processes, some reduction in *Salmonella* spp. numbers will occur. Thermisation processes range from 57 - 68°C for 15 seconds, and at the lower end of this range, little cell death will occur. At 60°C for 15 seconds a 2 log reduction is achievable and at 63°C a 4 log reduction is achieved (D'Aoust *et a*l., 1987).

Significant destruction of *Salmonella* spp. occurs during the curd cooking stage. Swiss Gruyere is cooked at 55 - 57°C for nearly an hour, then the pressed curd wheels (>20kg) are slowly cooled. At 55 - 57°C the D-value is between 2 - 9 minutes (ICMSF, 1996).

Some destruction of *Salmonella* spp. is expected for Swiss Emmentaler during curd cooking (52 - 53°C for 30 - 40 minutes). Bachmann and Spahr (1995) measured a 3 log reduction in numbers of *E. coli* directly after the curd was heated for 45 minutes at 53°C. Slow cooling of the pressed curd wheels (>70kg) also results in further death of *Salmonellae*.

The temperatures used in setting (30 - 32°C) and curd heating for the Swiss-type cheeses Appenzeller and Tilsiter (42 - 46°C), Tête de Moine (44 - 46˚C) and Vacherin Fribougeois (33 - 35°C) will permit the growth of any *Salmonellae* remaining after any initial heat treatment *e.g.* thermisation.

The number of viable *Salmonella* spp. cells will also decline during ripening and storage (Table 6). The rate of decline will be influenced by the temperature, water activity and pH of the cheese and will be accelerated by the presence of volatile fatty acids (Goepfert *et al*., 1968; Medina *et al*., 1982; White and Custer, 1976).

Table 6: Decrease in *Salmonella* spp. during ageing and maturation of cheese

Cheese	% Salt	pH	Log decrease	Ageing Conditions	Reference
Cheddar		5.4 - 5.65	2.5	26 weeks at 4.5°C	(Hargrove *et al*., 1969)
		5.2 - 5.3	5.3	26 wk at 4.5°C	
		5 - 5.05	5	13 wk at 4.5°C	
		5.2 - 5.4	4	13 wk at 10°C	
Cheddar			4	14 - 16 wk at 7.5°C	(Goepfert *et al*., 1968)
			4	10 - 12 wk at 13°C	
Cheddar	2.1 - 2.3	5.2	4.8 - 5.2	20 wk at 7°C	(Mehta and Tatini, 1994)
Samsoe		5.2	4	5 - 6 wk at 16 - 20°C + ca 3 wk at 10 - 12°C	(Goepfert *et al*., 1968)
Montasio		5.4 - 5.6	ca 4.5	12 - 13 wk at 12°C	(Stecchini *et al*., 1991)
Manchego	2.5 - 3	4.9 - 5.0	ca 7	8 wk at 10°C	(Medina *et al*., 1982)
	2.5 - 3	4.9 - 5.0	4.6 - ca 6.5	6 wk at 10°C	
Tilsiter	1.23	5.2 - 5.4	6.3	4 wk at 11 - 13°C	(Bachmann and Spahr, 1995)

Time and extent of decrease in *Salmonella*e in Cheddar is from 1 day after production; in the semi-hard cheeses, Montasio from day 3 (after brine-salting) and Tilsiter from day 3 (after brine-salting and 1 day ripening); and for Manchego, from day 2 (after brine-salting). Cheddar used by Mehta and Tatini, 1994 had an a_W of 0.95-0.97. Internal salt content of Manchego after 60 d, and for Tilsiter after 90 d.

Swiss Gruyere is ripened at 16°C for 20 - 40 days and at pH of 5.2 - 5.5. These conditions are likely to result in significant death of any contaminating *Salmonellae*. These cheeses must be held for a minimum of 120 days before sale. Prolonged storage would result in further die-off.

Swiss Emmentaler ripening conditions (Table 1) are likely to result in an estimated 3 log reduction in numbers of *Salmonellae* based on studies of similar cheeses (Table 6). After ripening, further death occurs during storage at 12°C for a minimum of 120 days before shipping. The volatile fatty acid content of Emmentaler (about 120mmol/kg) (Steffen *et al*., 1993) together with the pH of <5.7 are also likely to exert an additional bacteriocidal effect.

The manufacturing and ripening conditions for Appenzeller and Tilsiter cheese and physicochemical properties are similar. The effect of salt content, volatile fatty acids and water activity on the death rate of *Salmonella* spp. in Appenzeller and Tilsiter cheese is similar to that described for *E. coli*.

In Tilsiter-style cheese, a 6.3 log reduction in numbers of one strain of *Salmonella* spp. occurred during 4 weeks of ripening at 11 - 13°C (Bachmann and Spahr, 1995).

The extent of death of *Salmonellae* during ageing and storage of Swiss Tête de Moine cheese appears to be unknown. The lower water activity (0.93) compared to that of Tilsiter would indicate a faster decrease in viable *Salmonellae*, but the rising pH (5.2 to 5.6 - 5.8) during maturation would reduce the death rate. It is difficult to know the extent of this decrease as little information exists on the destruction of *Salmonella* spp. during the manufacture of this cheese.

Swiss Vacherin Fribougeois cheese is matured at 14 - 16°C for 30 - 42 days with a minimum age before shipping of 90 days. A shorter brining time (6 - 8 hr) and a water content of about 43%, suggest that the water activity is likely to be more than that of Tilsiter.

It is difficult to know how the extent of death of *Salmonellae* during ripening and storage of Swiss Vacherin Fribougeois cheese compares with that measured for other cheeses (Table 6). The lower pH profile (5.2 - 5.4) during this time would tend to give a faster death rate, but this may be offset by a higher water activity. From the data in Table 6, it is likely that the reduction in *Salmonella* spp. numbers over 90 days is not more than the 4 log reduction obtained in 10 - 12 weeks in Cheddar stored at 13°C.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
Gruyere	Some initial growth. Curd cooking result in death	Significant destruction of remaining *Salmonellae* at pH 5.2-5.5	Eliminates
Emmentaler	Some initial growth. Curd cooking result some destruction (3 log)	Likely further significant destruction	Eliminates
Appenzeller, Tilsiter	Some initial growth. Temperatures permit growth (100 fold)	A reduction of 4 logs expected in Appenzeller and 6.3 logs in Tilsiter	Eliminates
Tête de Moine		A reduction in > 5 logs expected	Eliminates
Vacherin Fribougeois		A reduction in ~ 4 logs expected	Eliminates

3.1.4 *S. aureus*

If *S. aureus* is present in raw milk it may grow during the initial stage of fermentation (Spahr and Url, 1994). Detectable levels of enterotoxin can be produced when the population reaches about 10^6 cfu/ml (ICMSF, 1998). For such populations to be attained there has to be growth of the organism before fermentation or slow production of acid. During cheese ripening, numbers of *S. aureus* will decline and viable cells may not be detectable at the time of consumption, however the enterotoxin will remain. *S. aureus* is inhibited by low pH and is a poor competitor; hence significant increases during cheesemaking reflect problems with the starter culture.

At the lower end of the thermisation temperature range (57 - 68°C), there will be little destruction of contaminating *S. aureus*, but significant reduction does occur at 64 - 68°C (Johnson *et al*., 1990b).

The heating temperatures used in curd formation for Gruyere cheeses (56 - 57°C for about 1 hour) will reduce the numbers of *S. aureus* by 2 log numbers (D-value at 54.5°C about 27 minutes: (ICMSF, 1996). The lethal effect of heating during Emmentaler production will be less (52 - 53°C for about one hour). Heating during curd formation of Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois will have little if any lethal effect on *S. aureus.*

However, since a slow fermentation rate is the prime cause of staphylococcal growth and enterotoxin formation, it is important that fermentation rates be fast and controlled. The pH reduction in Swiss-type cheeses is rapid (pH 5.5 in less than about 5 hours for Emmentaler, Tilsiter, Appenzeller, and Tête de Moine and pH 5.5 in about 8 hours for Gruyere).

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
All Swiss-type cheeses	Initial growth. Rapid acidification will limit growth. Curd cooking inEmmentaler and Gruyere decrease numbers.	Studies indicate limited survival after 1 day in hard Swiss cheese and not detectable after 90 days in semi-hard cheeses.	99% reduction

3.1.5 *Listeria monocytogenes*

The survival and proliferation of *L. monocytogenes* in Swiss-type cheeses is influenced by manufacturing conditions including heat treatment, fermentation and ripening.

During the initial stages (coagulation, curd heating and pressing) of manufacture of Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois, temperatures (30 - 46°C) permit growth of *L. monocytogenes* (ICMSF, 1996).

Thermisation times and temperatures ranging from 57 - 68°C for 15 seconds will have a variable effect on *L. monocytogenes*. The D-value for *L. monocytogenes* in milk is 33.3 seconds at 63.3°C and 7.0 - 7.2 seconds at 68.9°C (Johnson *et al*., 1990b). At the lower end of the temperature range for thermisation, there is little if any destruction of *L. monocytogenes.*

During the initial fermentation of Swiss Gruyere cheeses, the temperatures used during curd cooking (56 - 57°C for 1 hour) will reduce the numbers of any contaminating *L. monocytogenes* considerably (D-value at 52.2˚C is 24 - 37 minutes; D-value at 57.8°C is 4.4 - 5.2 minutes; (ICMSF, 1996).The lethal effect of heat during curd cooking in the manufacture of Emmentaler will be less (52 - 53°C for about 1 hour); however in the study by Bachman and Spahr (1995) no *L. monocytogenes* was detected in Emmentaler cheese one day after manufacture following an initial inoculation of 10^4 - 10^6 cfu/ml.

Even with rapid fermentation, entrapment and growth of *L. monocytogenes* in the curd during the first 9 - 10 hours could result in *L. monocytogenes* populations in cheese being 10 - 100 times that in the raw milk (Papageorgiou and Marth, 1989b; Ryser and Marth, 1987a; Yousef *et al*., 1988). At salting, the combination of low pH and decreased water activity stops growth of *L. monocytogenes*.

During the long ripening period (4 - 20 months) of Swiss-type cheeses, the numbers of any *L. monocytogenes* in the cheese would be expected to decline at a rate affected by pH, water activity and storage temperature (Papageorgiou and Marth, 1989b; Ryser and Marth, 1987a; Yousef *et al*., 1988). However, *L. monocytogenes* has been shown to survive and even grow on the external surfaces of cheese during ripening and maturation. The extent of survival on the surface is also dependent upon the pH of the cheese and at levels of ~ pH 5.5 no growth should occur on the outer surface of hard cheeses (Bachmann and Spahr, 1995).

It is unlikely that growth of *L. monocytogenes* would occur in Gruyere cheese due to the pH and water activity of ripened cheese (5.4-5.7 and 0.94, respectively). However, surface treatment with *Brevibacterium linens* may allow growth on the surface, particularly if the surface pH is 6 and above (Ryser and Marth, 1989a).

The pH of Emmentaler and water activity conditions after ripening (5.7-5.8 and 0.97, respectively) may permit growth of *L. monocytogenes* if there was contamination post curd cooking. Also for the moist crust variety, growth may occur at the surface because of its higher pH.

Bachmann and Spahr (1995) reported there is little change in numbers of *L. monocytogenes* contamination during 90 days maturation in Tilsiter cheese. In particular, surface growth of *L. monocytogenes* has been observed on Tilsiter-style cheese (Bachmann and Spahr, 1995). The pH rises to about 5.7 - 5.85 as a result of surface microbial activity and this improves survival of any contaminating *Listeria* spp. within the cheese.

In Appenzeller cheeses, the rise in pH from an initial pH of 5.2 mirrors that for Tilsiter and suggests surface pH values of more than 6 are reached during ageing. Thus, growth of any surviving *Listeria* spp. on or near the surface of Appenzeller and Tilsiter cheeses could occur. An initial decrease in numbers of *L. monocytogenes* has also been reported on immersion of semi-hard cheeses in brine followed by an increase during ripening with the final count of *L. monocytogenes* in cheese similar to that in the milk (Dominguez *et al*., 1987).

For Vacherin Fribougeois cheese, the pH appears to remain low (5.2 - 5.4) after 3 months. This suggests the surface pH remains low and *Listeria* spp. growth may not be a problem. For Tête de Moine, though the pH of the cheese rises from 5.2 to 5.8-5.9, the water activity is reported to be 0.93. Since this is close to the minimum water activity permitting growth of *L. monocytogenes*, it is unlikely that growth will occur on this cheese.

Summary

Cheese type	Manufacture	Ripening/Maturation	Overall Effect of Processing
Gruyere, Emmentaler*	Minimum 2 log unit reductions during curd cooking	Reduction during ripening and storage.	Eliminates
Appenzeller, Tilsiter	Growth during coagulation, curd cooking and pressing (10 - 100 fold).	Some reduction while pH is low <5.6. No growth, but survival in interior of cheese. Rapid growth on exterior	10-fold increase
Vacherin Fribougeois, Tête de Moine	Growth (10 - 100 fold) during coagulation, curd heating and pressing	pH remains low (5.2 - 5.4) during ripening, unlikely to grow water activity of0.93 unlikely to support growth	10-fold increase

* Studies indicate *L. monocytogenes* not detected beyond 1 day (Bachman and Spahr, 1995)

3.2. Consumption of Swiss-type cheeses in Australia

Data from the Australian National Nutrition Survey[40] (NNS) gives an indication of the percentage of the population who consume various types of cheese and the amount they consume.

[40] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24 hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

Swiss cheese is not a major food item consumed by Australians and is only consumed in small volumes. Consumption data from the NNS indicate only 0.3% of those surveyed consumed Swiss cheese with the average amount consumed being 39.7 g (Table 7). Table 7 shows data from the NNS on the Australian average daily consumption of Swiss cheese by gender and age. Consumption of Swiss cheese varies across all age groups; however consumption of this cheese is much lower in children and those aged 65+.

It cannot be assumed that this same proportion of the population would also consume raw milk cheese. However it is likely that those who will consume raw milk cheese, will not increase their cheese consumption, rather they will substitute consumption of pasteurised cheese with raw milk cheese.
Swiss-type cheeses

Table 7: Australian average daily consumption of Swiss-type cheese by gender and age (Australian Government Department of Health and Family Services, 1997)

Gender	Age	No. of respondents	No. of consumers (% of respondents)	Average amount of cheese consumed (g/day)
Male	2 - 3	170	-	-
Male	4 - 7	416	1 (0.2)	5
Male	8 - 11	385	3 (0.8)	23
Male	12 - 15	349	-	-
Male	16 - 18	215	1 (0.5)	151
Male	19 - 24	485	2 (0.4)	49
Male	25 - 44	2140	18 (0.8)	39
Male	45 - 64	1554	13 (0.8)	33
Male	65+	902	-	-
Female	2 - 3	213	1 (0.5)	25
Female	4 - 7	383	2 (0.5)	15
Female	8 - 11	354	1 (0.3)	30
Female	12 - 15	304	-	-
Female	16 - 18	218	-	-
Female	19 - 24	575	9 (1.6)	26
Female	25 - 44	2385	20 (0.8)	24
Female	45 - 64	1752	15 (0.9)	28
Female	65+	1058	1 (0.1)	68

4 Risk Characterisation

In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles (Appendix 1).

The qualitative framework considers the characterisation of identified hazards (hazard identification and characterisation combined) and an assessment of the likely exposure to these hazards (exposure assessment) which when combined provides a characterisation of the risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of disease. The exposure module characterises exposure to the hazard based on the likely level of the hazard in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of the hazard on a "per serve" basis[41]. Essentially the matrix categorises the risk for each hazard by combining

[41] "per serve" is defined as the amount of product consumed per eating occasion.

information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

The model was employed to characterise the risk from selected Swiss-type cheeses.

Risk categories for hazards in Swiss-type raw milk cheeses were assigned as follows:

Hazard	Infective dose	Consequence of exposure	Severity of hazard
C. jejuni	100 - 1,000	Moderate/Serious#	Very Low/Low#
E. coli (EHEC)	<10	Serious	High
Salmonella spp.	10 - 100	Moderate/Serious#	Low/Moderate#
S. aureus	>1,000	Mild	Negligible
L. monocytogenes	>1,000/10 - 100#	Moderate/Severe#	Negligible/Moderate#

susceptible populations

Exposure categories for Swiss-type raw milk cheeses were assigned as follows:

Hazard	Raw product contamination	Effect of processing	Exposure
C. jejuni	Infrequent (1%)	Eliminates	Negligible
E. coli (EHEC)	Infrequent (1%)	Eliminates	Negligible
Salmonella spp.	Infrequent (1%)	Eliminates	Negligible
S. aureus	Sometimes (10%)	Eliminates	Negligible
L. monocytogenes	Infrequent (1%)	Eliminates/10 fold increase*	Negligible/Moderate*

* Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine cheeses

Risk characterisation for raw milk Swiss-type cheese:

Hazard	Hazard Characterisation	Exposure Assessment	Risk Characterisation
C. jejuni	Very low/low#	Negligible	Negligible
E. coli (EHEC)	High	Negligible	Low
Salmonella spp.	Low/Moderate#	Negligible	Negligible/Very Low#
S. aureus	Negligible	Negligible	Negligible
L. monocytogenes	Negligible/Moderate#	Negligible/Moderate*	Negligible/Very low# Low*/High*#

Susceptible populations
* Appenzeller, Tilsiter, Vacherin Fribougeois and Tête de Moine cheeses

5 Conclusions

The Swiss-type raw milk cheeses Gruyere, Emmentaler, Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois have been assessed as having a negligible to low likelihood of contamination with pathogenic microorganisms such as *E. coli, S. aureus, Salmonella* spp. and *Campylobacter.* Although these organisms may be present in the raw milk and grow during initial stages of cheese manufacture, the processing conditions and physicochemical properties of the cheeses are not conducive to the growth or survival of these organisms. The possibility exists that *L. monocytogenes* may grow and/or survive in Swiss Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses and therefore pose a high risk if consumed by susceptible individuals.

The presence of *S. aureus* in raw milk poses a risk to all Swiss-type raw milk cheeses if conditions permit growth to levels sufficient to produce enterotoxin, as once formed, the cheesemaking process will not inactivate the toxin. Rapid acidification (pH 5.5 in less than 5hrs or 8hrs in Gruyere) effectively prevents growth of *S. aureus* and enterotoxin production.

Although some Swiss-type raw milk cheeses *e.g.* Appenzeller, Tilsiter and Vacherin Fribougeois, may be produced from thermised milk, thermisation will have a variable effect on destroying pathogenic microorganisms. At the lower end of the thermisation temperature range there will be little, if any, lethal effect. High temperatures(52 - 58^{o}C) used in curd cooking for the manufacture of Swiss Gruyere and Emmentaler cheeses results in considerable destruction of pathogens. Further reduction in numbers is estimated to occur during the long ripening/maturation period and storage conditions (pH, temperature and water activity).

Low curd cooking temperatures (33 - 46^{o}C) used in the manufacture of Swiss Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois, will not reduce numbers of pathogenic organisms and may permit growth up to 100-times that present in the raw milk. Therefore reduction in levels of pathogenic organisms in these cheeses is reliant on temperature, pH and water activity during ripening/maturation and storage.

Bachmand and Sphar (1995) assessed the safety of Swiss-type hard (Emmentaler) and semi-hard (Tilsiter) cheeses made from raw milk. *C. jejuni, E. coli, L. monocytogenes, Salmonella* spp. and *S. aureus* were not detected beyond 1 day in Emmentaler cheese. Pathogens were found to survive longer in the Tilsiter cheese but after 90 days of ageing at 11-13^{o}C, all pathogens except *L. monocytogenes* were below detectable limits. *Listeria* spp. may survive in the interior and surface of Swiss-type semi-hard cheeses, with growth on the surface possible as the pH increases during ripening.

There is a degree of uncertainty surrounding the fate of pathogens in Swiss Gruyere, Emmentaler, Tête de Moine and Vacherin Fribougeois cheeses. Although challenge studies are available for Emmentaler and Tilsiter cheese, no data is available on the other Swiss-type cheeses. The safety of these cheeses has therefore been assessed on the likely effect cheesemaking has on pathogens, based on processing (curd cooking and maturation conditions) and intrinsic chemical characteristics (such as pH, water activity and moisture content).

Although staphylococcal intoxication has been attributed to Swiss-type style cheeses (*e.g.* Emmentaler and Gruyere) there have been no reported outbreaks of foodborne illness from pathogenic microorganisms such as *E. coli, Salmonella* spp., *Campylobacter* spp. and *L. monocytogenes* in Swiss-type raw milk cheese.

The process of manufacturing selected Swiss-type raw milk cheeses has been assessed to affect selected pathogens as follows:

Pathogen	Risk associated with raw milk Swiss-type cheese
Campylobacter spp.	*Campylobacter* spp. are unlikely to survive processing and maturation and are a **negligible** risk.
E. coli (EHEC)	**Low** risk as the organism doesn't survive the curd cooking process in the high curd cook cheeses or cheese maturation.
Salmonella spp.	**Negligible** risk (general population) to **Very Low** risk (susceptible population) as the organism doesn't survive the curd cooking process in the high curd cook cheeses or cheese maturation.
S. aureus	Risk from *S. aureus* is considered **negligible**. Conditional on good control over animal health and raw milk handling. The organism doesn't survive the cooking process.
L. monocytogenes	**Low** risk for general population. **High** risk for susceptible populations in Swiss Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses. *L. monocytogenes* does not survive the manufacturing process for Sbrinz, Gruyere and Emmentaler cheeses however may grow in the initial stages in manufacturing and survive maturation in Appenzeller, Tilsiter, Tête de Moine and Vacherin Fribougeois cheeses.

There is no difference in the public health and safety risk from any of the selected pathogens in selected Swiss-type raw milk cheeses made from cow, goat or sheep milk.

The findings of the raw milk Swiss-type cheeses assessed may be applied to other Swiss-type cheese as this group of bacterially ripened cheeses with eyes have similar physicochemical characteristics and manufacturing protocols. However the raw milk cheeses assessed cannot be applied to other hard cheeses based on moisture (*i.e.* 37 - 42% moisture) as the moisture content of the cheeses assessed (34 - 44%) overlap between the extra hard and hard moisture categories and do not represent all types of hard cheeses in respect to physicochemical characteristics and manufacturing protocols.

Appendix 10: Risk assessment – Cheddar cheese

1 Introduction

Cheddar cheese is a dry-salted hard cheese (Fox *et al*., 2000; Ottogalli, 2000a; Ottogalli, 2000b; Ottogalli, 2001; Scott, 1986). Hard cheeses generally have a moisture content in the range 30 - 35% and are subjected to high pressure during manufacture to give a hard, uniform, close texture.

Cheddar cheeses generally undergo a curd cooking step at 37 - 39°C, followed by cheddaring the drained curd which allows ongoing acid development within the curd mass. When the curd reaches pH 5.4 the Cheddar blocks are milled and dry salted. Cheddar cheese may ripen without temperature control or in a controlled environment at 4 - 8°C for periods ranging from 4 months to greater than 2 years.

The Codex standard for Cheddar cheese[42] contains details on the principal characteristics of this cheese (such as appearance, texture and origin of milk) and specifies a maximum moisture content of 39%.

This risk assessment examines the fate of *Escherichia coli* (EHEC), *Staphylococcus aureus* and *Listeria monocytogenes* during the manufacture of a raw milk Cheddar cheese based on a probabilistic model developed by the University of Tasmania and adapted by FSANZ.

The manufacturing parameters and physicochemical properties for the modelled raw milk Cheddar cheese are based on experimental data and do not necessarily reflect commercial manufacturing practices. The modelled raw milk Cheddar cheese manufacturing parameters and physicochemical characteristics are described in Figure 1.

A qualitative framework was subsequently used to rate the risk to public health and safety from the consumption of raw milk Cheddar cheese made from cow, goat or sheep milk, containing these microbiological hazards.

2 Hazard identification and hazard characterisation

In evaluating the safety of Cheddar cheese, the following pathogens were considered: *E. coli*, *L. monocytogenes*, and *S. aureus*. A detailed characterisation of potential hazards is attached as Appendix 14.

[42] Codex International Standard for Cheddar, *CODEX STAN C-1-1966*

3 Exposure assessment

The production stages in the manufacture of Cheddar cheese were modelled following the conditions and steps described by Reitsma and Henning (1996). A summary of the steps and the time and temperature conditions used is illustrated in Figure 1. Approximately 10 litres of milk produces 1kg of Cheddar cheese (Dairy Australia, 2004). A summary of input variables that are represented by distribution functions is shown in Table 1.

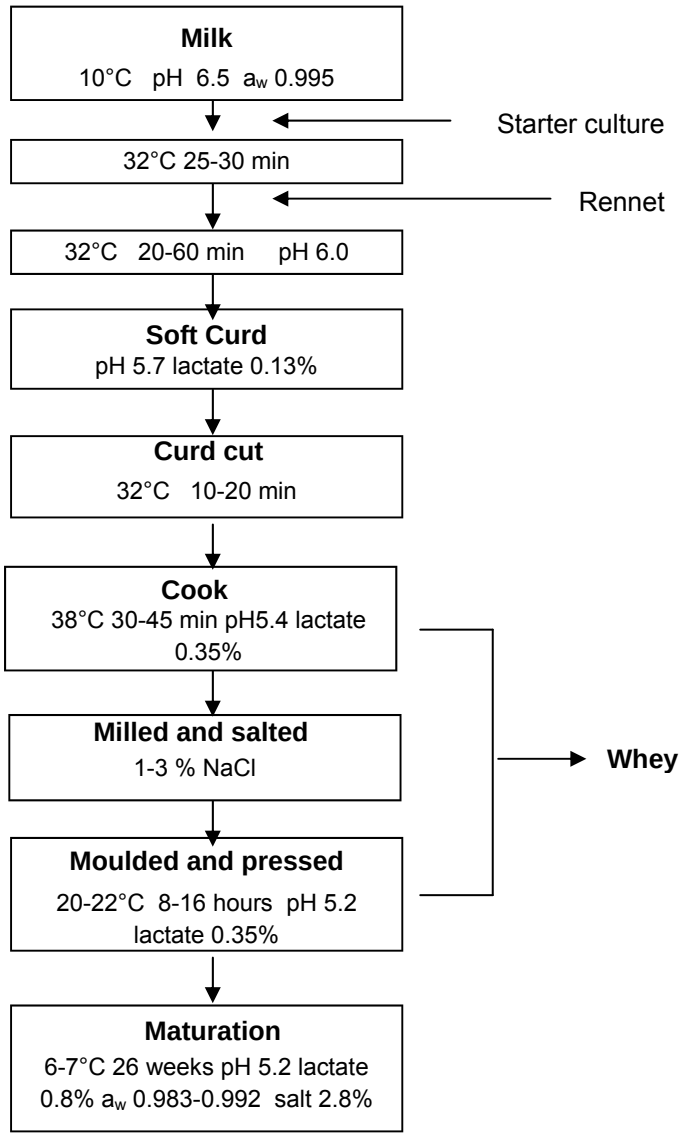

Figure 1: Conceptual model of Cheddar cheese production

Table 1: Input distribution functions used in the models for Cheddar cheese

Cheese type	Step	Input Parameter	Distribution
Cheddar cheese	Starter culture added	Time, minutes	Uniform (20, 30)
	Rennet added	Time, minutes	Uniform (20, 60)
	Curd Cut	Time, minutes	Uniform (10, 20)
	Curd Cooked	Time, minutes	Uniform (30, 45)
	Milled and salted	Salt, %	Uniform (1.5, 3)
	Moulded and pressed	Temperature, ºC	Uniform (20, 22)
		Time, hours	Uniform (8, 16)
	Maturation	Temperature, ºC	Uniform (6, 7)

3.1 Initial phase of manufacture

During the production of cheese, the cells of bacterial pathogens concentrate within the curd matrix as they form. Some cells are lost in the whey, however, this is only a small fraction of the total number. Yousef and Marth (1988) show that during the production of Colby cheese, between 1 - 3% of *L. monocytogenes* inoculated into milk used for cheesemaking were excluded in the whey. However there was a greater than 1 log increase in numbers during manufacture, outweighing any loss in the whey. It is not clear if this was due to growth, concentration during curd formation or a combination of both. Buazzi *et al.* (1992) found that during the production of Swiss cheese the concentration of *L. monocytogenes* in the curds was at least 20 times higher than that in the whey. This value increased to over a 100 fold higher concentration in curds during pressing of the cheese. During the production of parmesan cheese the ratio of *L. monocytogenes* between the curds and whey was between 18:1 and 324:1 (Yousef and Marth, 1990). The higher ratio observed by Yousef and Marth (1990) may be due to thermal inactivation during the higher cheese cooking temperature required for Parmesan cheese.

It is assumed in the model that losses of pathogens into the whey during production are insignificant, the pathogens are concentrated in the curds and that growth may occur during production.

3.2 Ripening

During the maturation/ripening of cheese it has been demonstrated that there is a decrease in the level of some pathogenic organisms (Bautista and Kroll, 1988; Ryser and Marth, 1987a; Schaffer, 1995). The rate of inactivation is dependent on the cheese variety, the pathogen and the temperature. A survey of scientific literature was undertaken to find data relevant to the cheeses being modelled. A description of the inactivation models follows.

It should be noted that cheese production and ripening conditions used in challenge studies may not represent those used for the production of Cheddar cheeses made in Australia. These model cheeses are, in some cases, produced with high moisture levels (and low salt concentrations) to promote the survival of pathogenic microorganisms and therefore represent a 'worst-case' situation. Reitsma and Henning (1996) for example produced Cheddar cheeses with salt levels of between 1.09 to 1.43% to ensure that the salt in moisture phase would be favourable for the growth of *E. coli*.

Inactivation rates

Survival of bacteria in cheese depends on a range of intrinsic and extrinsic factors. Intrinsic factors that may influence the survival of bacteria in cheese include the pH, moisture and chemical properties, such as organic acids (lactic and acetic acid), free fatty acids and other inhibitory metabolites of the starter cultures. A important extrinsic factor is the storage temperature.

As a first approximation, survival kinetics is described using a first-order inactivation equation:

$$\log_{10}\frac{N}{N_0} = -\frac{t}{D}$$

where N is concentration at time t (days), N_0 is the initial concentration and D is the decimal reduction time (days). The D-value is the time required for the bacterial population to decrease by a factor of ten.

3.2.1 Effect of ripening on *E. coli*

During the maturation phase the physicochemical properties of the curd are not conducive to the growth of *E. coli*. The low temperature combined with salt and low pH act in an inhibitory manner. It has been demonstrated that these conditions cause a gradual inactivation of *E. coli* (Bautista and Kroll, 1988; Reitsma and Henning, 1996; Schlesser *et al*., 2006).

Figure 2 presents a summary of the inactivation of *E. coli* from Schlesser *et al*. (2006). Each column represents a different initial concentration in the raw milk (10^1, 10^3 and 10^5 cfu/ml) while each row within a column is a different trial. In each case the survival of the *E. coli* follows first order inactivation kinetics. The straight lines in each panel are the least squares regression fit to the data. There appears to be little variation between trials for these data.

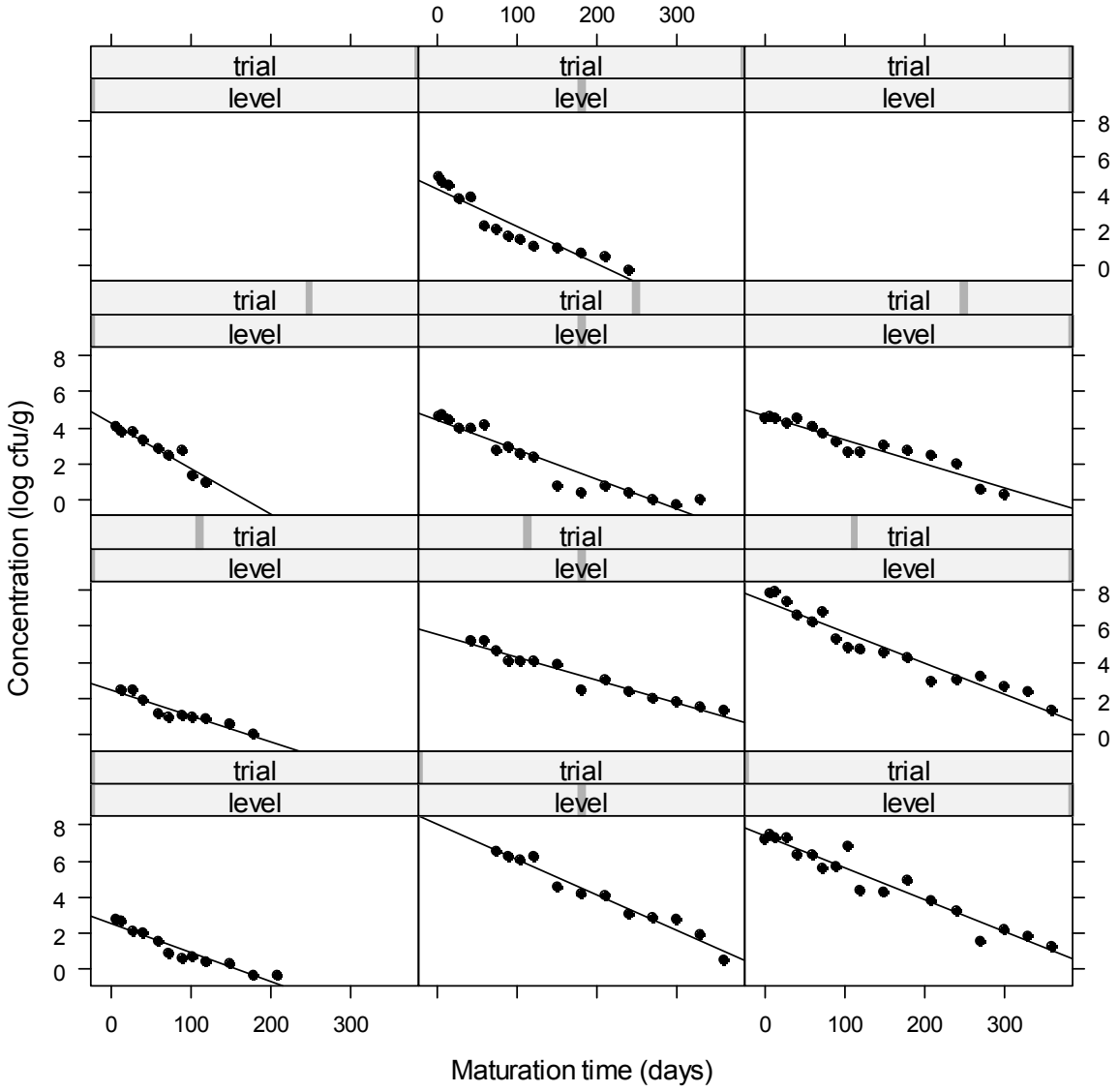

Figure 2: Inactivation of *E. coli* during Cheddar cheese maturation (from Schlesser *et al.*, 2006).

Similar analysis of data from Reitsma and Henning (1996) shows much greater variability between trials. Analysis of variance of the logarithm of the inactivation rates for these two data sources showed no statistical differences. As a result the inactivation rates from the two sources were pooled to develop a single distribution for the decimal reduction time.

Temperature affects were not considered in the development of the predictive model for the inactivation of *E. coli* during Cheddar ripening due to a lack of data.

3.2.2 Effect of ripening on *S. aureus*

During the ripening phase the temperature is too low to allow the growth of *S. aureus* and it is assumed that gradual inactivation occurs. A single published report showing the rate of inactivation of *S. aureus* during maturation of Cheddar cheese was found (Bautista and Kroll, 1988). Lindqvist *et al.* (2002) did not consider inactivation to be important for *S. aureus,* however, they were examining an unripened cheese in their risk assessment.

The inactivation model for *S. aureus* in the Pathogen Modelling Program, as described by Whiting *et al.* (1996), was used to model the inactivation rate of *S. aureus* during Cheddar cheese maturation. This model estimates the time to a 4-decimal reduction in numbers for *S. aureus* in a liquid broth culture and contains parameters for temperature (T), pH (P), salt concentration (S), lactate concentration (L) and nitrite concentration (N). As there is no nitrite added during cheese production (N = 0 ppm) the model has been modified to exclude nitrite terms (Equation 1).

$$\begin{aligned}\text{Log}_{10}(\text{T4D}) = {} & -3.742 + 0.03138T + 2.237P + 0.01128S - 0.1598L + 0.001674TP - \\ & 0.000407TS - 0.02318TL + 0.002015PS - 0.1564PL + 0.03187SL - 0.001225T^2 - \\ & 0.1629P^2 - 0.001988S^2 + 0.6158L^2\end{aligned}$$

Equation 1

A comparison between the inactivation rate predicted by the Whiting *et al.* (1996) model and the inactivation observed by Bautista and Kroll (1988) is shown in Figure 3. Confidence intervals shown are for the data of Bautista and Kroll (1988). Model parameters values that most closely matched the experimental conditions used by Bautista and Kroll (1988) are as follows: temperature: 12ºC, pH: 5.4, salt concentration: 2% (w/w), lactate concentration – 0.75% (w/w). The lactate concentration value used is from Chou *et al.* (2003) as there was no value given in Bautista and Kroll (1988).

The model of Whiting *et al.* (1996) provides a conservative estimate of the inactivation of *S. aureus* during maturation of Cheddar cheese and more accurately predicts the numbers of *S. aureus* in the later stages of maturation than the early stage. However, Whiting *et al*. (1996) reports that the inactivation of *S. aureus* in broth medium does not follow first order kinetics and as a result the predictions using this model may not be accurate beyond 4 log reductions.

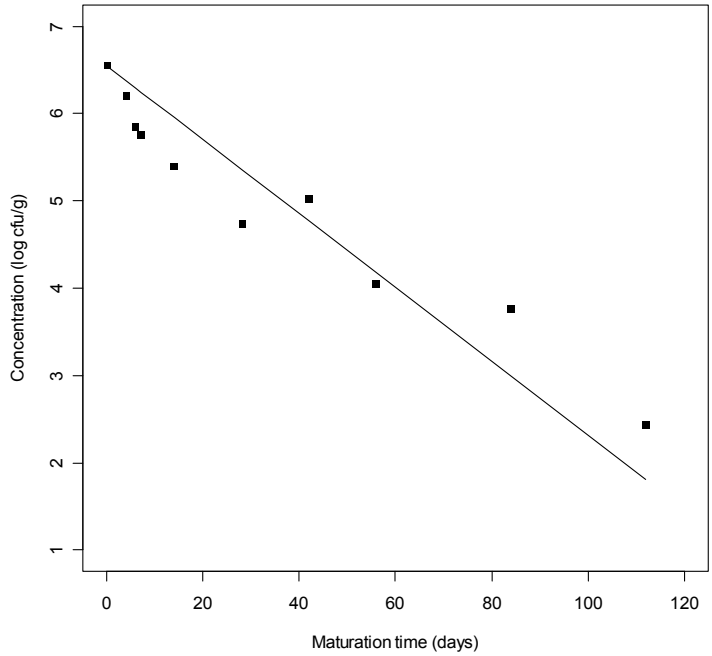

Figure 3: Comparison between experimental data for *S. aureus* counts in Cheddar cheese maturation (Bautista and Kroll, 1988) and inactivation model (Whiting *et al*., 1996).

3.2.3 Effect of ripening on L. monocytogenes

From the literature, the concentration of *L. monocytogenes* in cheese during maturation shows an increase in the first two weeks of ripening followed by a decline during subsequent weeks. This has been demonstrated in Cheddar, Manchego and Swiss-type cheeses (Figure 4) (Buazzi *et al*., 1992; Buyong *et al*., 1998; Dominguez *et al*., 1987; Ryser and Marth, 1987b).

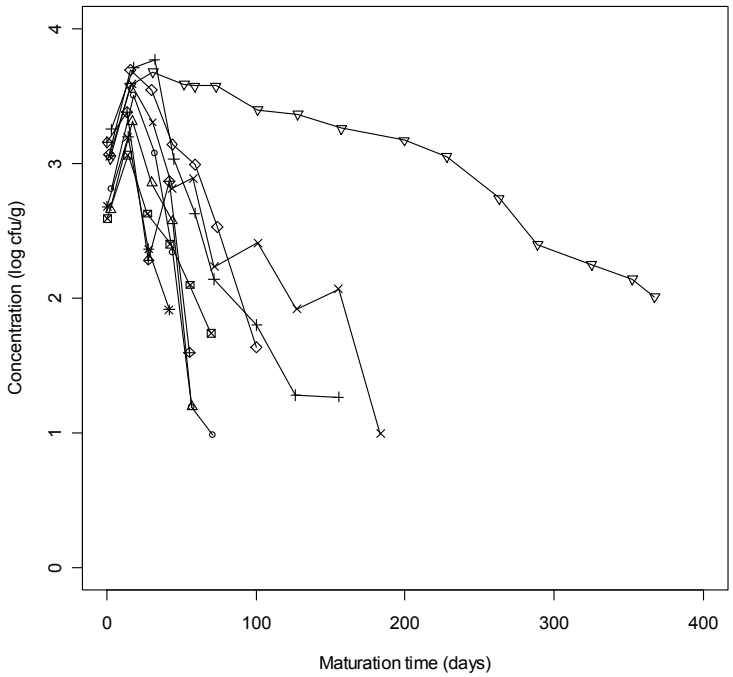

Figure 4: Effect of maturation on the concentration of various strains of *L. monocytogenes* in Cheddar cheese (adapted from Ryser and Marth, 1987b).

Ryser and Marth (1987b) provided the most comprehensive data for the effects of Cheddar cheese maturation on *L. monocytogenes*, comparing three different strains under different conditions. The strains were Scott A serotype 4b a clinical isolate, V7 serotype 1 a milk isolate, and CA serotype 4b an isolate from Mexican style cheese. The fate of these three strains in Cheddar cheese when matured at 6ºC and 13ºC is shown (Figures 5 and 6). The initial increase in numbers in the figures is accounted for by growth during the manufacture of the cheese.

A decimal reduction value (D-value) was calculated for each of the inactivation curves for *L. monocytogenes* in Cheddar cheese from Ryser and Marth (1987b). This approach has been used to determine the effect of maturation on the survival of *L. monocytogenes* in cheese previously (Yousef and Marth, 1990). The decimal reduction times were determined from the least squares regression fit to the survival data (Figures 5 and 6).

Unexpectedly there was no difference in the inactivation rates of *L. monocytogenes* for Cheddar cheese stored at 6°C and 13°C. As a result, temperature was not included in the predicted model. A normal distribution was fitted to the logarithm of the D-values for matured cheese at 6ºC.

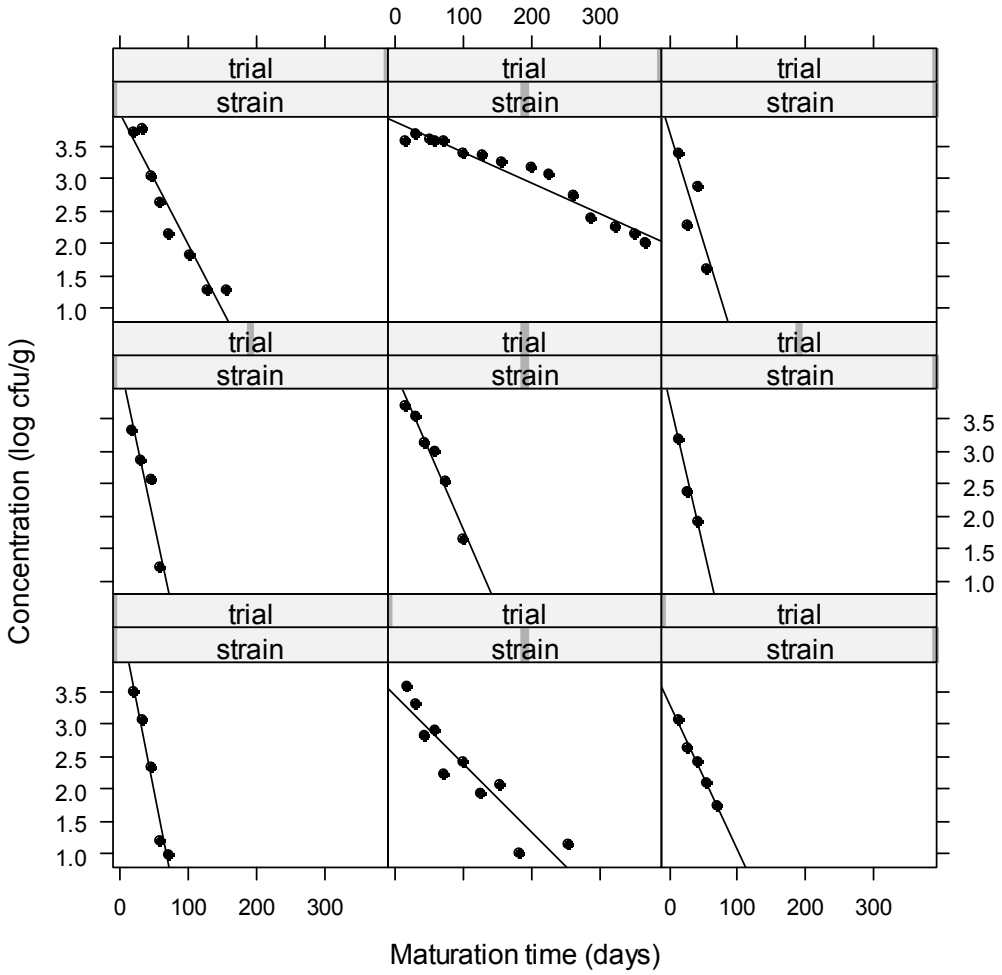

Figure 5: Fate of *L. monocytogenes* during Cheddar cheese maturation at 6ºC (adapted from Ryser and Marth, 1987b).

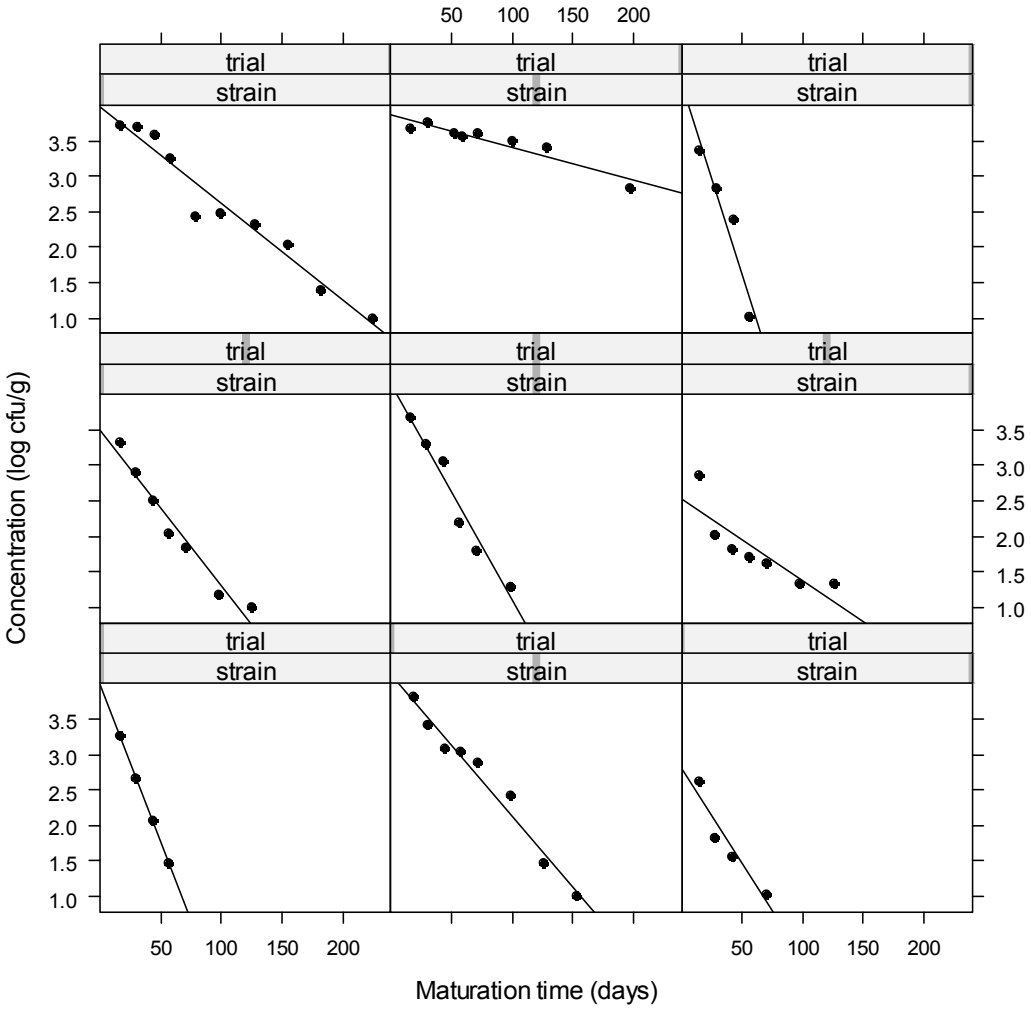

Figure 6: Fate of *L. monocytogenes* during Cheddar cheese maturation at 13 ºC (adapted from Ryser and Marth, 1987b).

The three rows in each columns present results from different trials of the *L. monocytogenes* strains Scott A, V7 and CA, respectively. The straight lines in each panel are the least squares regression to the experimental data.

For the survival of *L. monocytogenes* in Cheddar cheese, the results of Ryser and Marth (1987b) highlight the variability in inactivation rates both between strain and between trials. This is most clearly seen in the results for strain V7 (middle column in Figures 5 and 6). Decimal reduction times at 13 °C range between 32.5 - 218.9 days for trials 2 and 3, respectively. Similar results were found for survival at 6 °C.

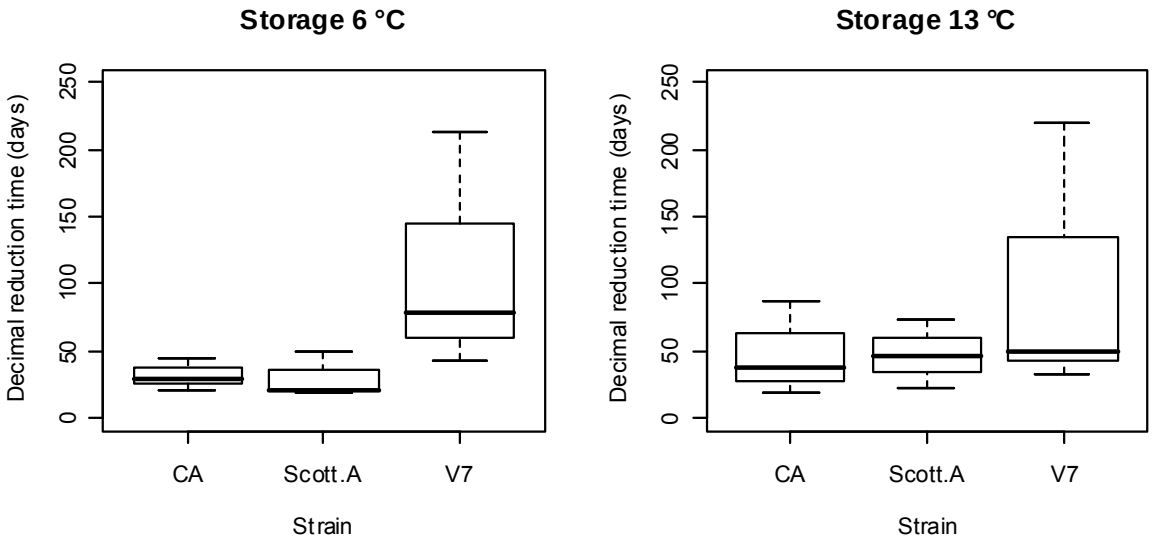

Figure 7: Boxplots of decimal reduction times (days) for *L. monocytogenes* strains California (CA), Scott A and V7 in Cheddar chesses stored at 6 and 13 °C.

3.3 Probabilistic model results

Results of the probabilistic models suggest that there is substantial growth of the three pathogenic bacteria during the production of Cheddar cheese. This is due to the temperatures required during activation and cooking (about 32ºC) and the long time required for pressing of the curd to remove the whey. These temperatures are conducive to the growth of pathogens.

The amount of growth modelled for each of the three pathogens was substantial with *E. coli* having the greatest amount of growth (4.13 logs). *S. aureus* was found to have the lowest concentration (-4.69 logs) at the end of ripening with *E. coli* having the greatest concentration (+0.98 logs). The main difference in the predicted concentrations at the end of ripening was due to differences in the decimal reduction times for survival. This was reflected in the sensitivity analysis for each pathogen that showed that the decimal reduction time is the most important factor for influencing the concentration at the end of ripening. Other factors such as the temperature and time for pressing the cheese curd were of lesser importance.

In order to assess the combined effect of production and ripening on the pathogen concentrations, the model predictions for *E. coli* and *L. monocytogenes* were compared to the *Australia New Zealand Food Standards Code* (the Code).

For *E. coli* and *L. monocytogenes* the concentration of the organism per gram in the matured raw milk cheese is of importance for compliance with the Code. For *S. aureus* the maximum concentration that the organism reached at any stage during the production of the raw milk cheese was of importance. A concentration between 10^5/g and 10^6/g was considered indicative of toxin production from *S. aureus* (FDA, 2003; Lindqvist *et al*., 2002). The maximum concentration reached by any of the organisms in the model was predicted to occur at a point just prior to commencement of maturation.

3.3.1 *E. coli*

A summary of the simulation results for *E. coli* in raw milk Cheddar cheese is provided in Table 2. For raw milk Cheddar cheese made from milk containing 0.001 cell/ml of *E. coli* the maximum concentration that the organism will reach at any stage during raw milk Cheddar cheese production is estimated to be 1.88×10^{4}/g. The estimated concentration in raw milk Cheddar cheese matured for 26 weeks (6 months) ranges between 1.80×10^{-7} cells/g and 31.8 cells/g with a mean of 44.5/g. Increasing the initial contamination level in milk for cheese production proportionally changes the mean estimated concentration in cheese prior to maturation, *i.e.* a 1 log higher initial contamination results in a 1 log higher mean contamination at the completion of maturation. Raw milk Cheddar cheese made from raw milk containing 0.1 cell/ml of pathogenic *E. coli* reached a maximum population of 6.48×10^{5} cells/g during cheese production. The contamination in the finished product was predicted to range between 1.77×10^{-5} cells/g and 2.46×10^{3} cells/g with a mean of 8.48 cells/g.

The microbiological limit in the Code for *E. coli* in cheese specifies that 4 of 5 samples must have less than 10 cfu/g, with no single sample having greater than 100 cfu/g. Based on the model estimates, cheese made with raw milk with an initial contamination of greater than 1 cell/ml will fail to meet the specified testing requirements in approximately 50% of cases (Figure 8).

Table 2: Estimates of *E. coli* concentration in raw milk Cheddar cheese made from milk with different initial contamination levels

	Initial contamination in milk				
	0.001 cell/ml	0.1 cells/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	3.79×10^{-1}	2.88×10^{1}	3.26×10^{2}	2.97×10^{3}	3.41×10^{4}
Mean	4.45×10^{1}	4.73×10^{3}	4.21×10^{4}	4.36×10^{5}	4.51×10^{6}
Maximum	1.88×10^{4}	6.48×10^{5}	5.34×10^{6}	8.33×10^{7}	1.73×10^{9}
5th percentile	1.85×10^{0}	1.90×10^{2}	1.81×10^{3}	1.83×10^{4}	1.87×10^{5}
95th percentile	1.60×10^{2}	1.67×10^{4}	1.57×10^{5}	1.60×10^{6}	1.59×10^{7}
End of 6 month maturation concentration in cheese (cells/g)					
Minimum	1.80×10^{-7}	1.77×10^{-5}	5.31×10^{-4}	8.65×10^{-3}	1.05×10^{-2}
Mean	7.77×10^{-2}	8.48×10^{0}	8.47×10^{1}	7.43×10^{2}	7.82×10^{3}
Maximum	3.18×10^{1}	2.46×10^{3}	2.85×10^{4}	2.63×10^{5}	4.43×10^{6}
5th percentile	2.85×10^{-4}	3.04×10^{-2}	2.75×10^{-1}	2.77×10^{0}	2.91×10^{1}
95th percentile	2.86×10^{-1}	3.01×10^{1}	2.91×10^{2}	2.78×10^{3}	2.79×10^{4}

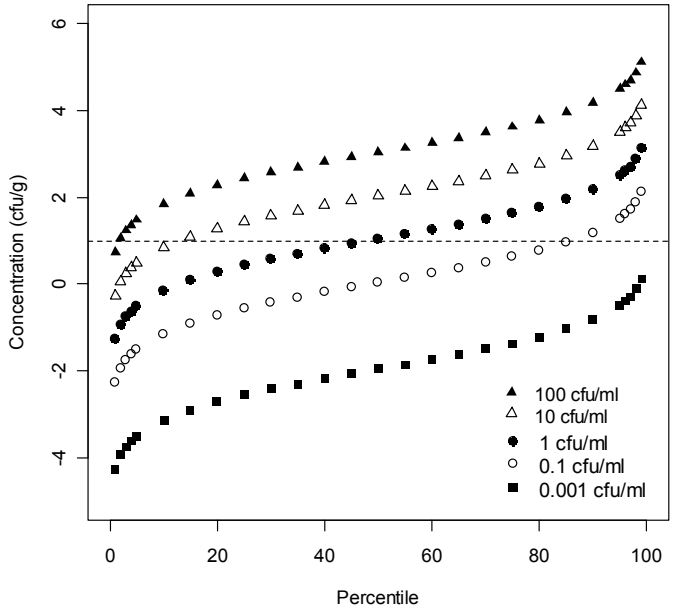

Figure 8: Estimated concentration of *E. coli* in raw milk Cheddar cheese made from milk with different initial contamination at the end of 26 weeks maturation

3.3.2 *S.aureus*

During the manufacture of raw milk Cheddar cheese there is the potential for *S. aureus* to grow. Figure 9 shows the changes in concentration of *S. aureus* during Cheddar cheese production made from milk with an initial concentration of 100 cfu/ml. The boxes in the figure indicate the 25th and 75th percentile values of the iterations for the concentration of *S. aureus* at each stage in production.

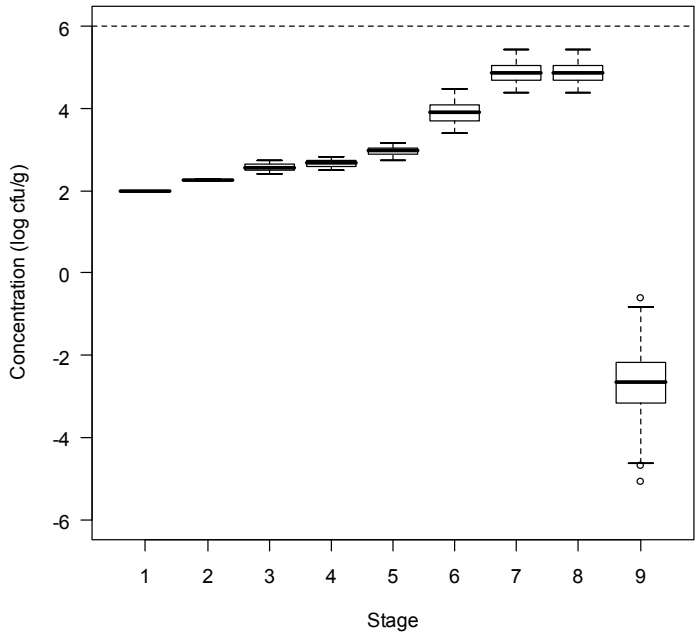

Figure 9: **Box and whisker plot of** *S. aureus* concentration during raw milk Cheddar cheese production. Initial contamination of milk of 100 cfu/ml. Stage numbers from Figure 1. Stages are from raw milk (Stage 1) through production to the end of maturation (Stage 9).

An initial contamination of 100 cells/ml in raw milk entering the Cheddar cheese production process resulted in a maximum concentration of 4.92×10^5 cfu/g of cheese at the start of maturation (Figure 9), while the maximum concentration reached with an initial contamination of 10 cfu/ml in raw milk was 4.87×10^4 cfu/g. After maturation these maximum concentrations had fallen to 1.25 cfu/g and 2.51×10^{-1} cfu/g, respectively. In no simulation was the threshold of 10^6/g adopted by Lindqvist *et al.* (2002) reached, however, if the lower value of 10^5 was used (FDA 2003) then cheese made from raw milk with a concentration of *S. aureus* greater than 10 cfu/ml may exceed this threshold during the initial fermentation phase. Lower initial contamination concentrations of *S. aureus* in raw milk result in maximum cell concentrations below the threshold population level for toxin production.

The prediction that *S. aureus* concentrations may exceed the thresholds does not mean that toxin would be produced with resulting illnesses. Heterogeneity in toxin production between strains and the time for toxin production would also need to be considered. This is outside the scope of the current work, however the predictions suggest that *S. aureus* concentrations would need to be well above 100 cfu/ml in raw milk before toxin production is likely to occur in a large proportion of fermentated milk samples.

Table 3: Estimates of *S. aureus* concentration in raw milk Cheddar cheese made from milk with different initial contamination levels

	Initial contamination in milk				
	0.001 cell/ml	0.1 cell/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	1.73×10^{-1}	1.74×10^{1}	1.71×10^{2}	1.59×10^{3}	1.67×10^{4}
Mean	8.51×10^{-1}	8.45×10^{1}	8.40×10^{2}	8.45×10^{3}	8.46×10^{4}
Maximum	5.02×10^{0}	4.78×10^{2}	4.23×10^{3}	4.87×10^{4}	4.92×10^{5}
5th percentile	3.21×10^{-1}	3.21×10^{1}	3.16×10^{2}	3.16×10^{3}	3.21×10^{4}
95th percentile	1.84×10^{0}	1.82×10^{2}	1.81×10^{3}	1.84×10^{4}	1.82×10^{5}
End of 6 months maturation concentration in cheese (cells/g)					
Minimum	3.53×10^{-12}	7.91×10^{-12}	8.84×10^{-10}	1.01×10^{-8}	4.09×10^{-7}
Mean	1.05×10^{-7}	1.07×10^{-5}	1.08×10^{-4}	1.04×10^{-3}	1.05×10^{-2}
Maximum	9.91×10^{-6}	1.60×10^{-3}	1.50×10^{-2}	2.51×10^{-1}	1.25×10^{0}
5th percentile	7.49×10^{-10}	7.10×10^{-8}	7.27×10^{-7}	7.15×10^{-6}	7.54×10^{-5}
95th percentile	4.41×10^{-7}	4.36×10^{-5}	4.37×10^{-4}	4.27×10^{-3}	4.37×10^{-2}

3.3.3 *Listeria monocytogenes*

Model estimates for raw milk Cheddar cheese matured for 26 weeks gave highly variable levels of *L. monocytogenes* in the final product (Table 4). The variability is primarily due to differences in inactivation during maturation between strains. The minimum estimated concentration of *L. monocytogenes* in finished product was $< 10^{-14}$ cell/g from milk contaminated with 10^{-3} cell/ml. The maximum modelled concentration of *L. monocytogenes* in matured raw milk Cheddar was 2.68×10^5 cells/g from raw milk containing 10^2 cells/ml. The mean estimated concentration of *L. monocytogenes* in matured raw milk Cheddar cheese ranged between 1.50×10^{-2} - 1.50×10^3 cells/g. A summary of the estimated concentration of *L. monocytogenes* in raw milk Cheddar cheese is presented in Table 4.

The microbiological limit in the Code for *L. monocytogenes* in cheese requires that the organism not be detectable in five samples of 25g. The presence of a single cell in 125g of Cheddar cheese indicates a breach of the Code. An initial contamination of 10^{-3} cells/ml in milk met the regulatory limit in 81% of cases, while raw milk Cheddar cheese made from

milk contaminated with 0.1 cell/ml of *L. monocytogenes* met the regulatory limit in 49% of cases. For raw milk cheeses made from milk with 1, 10 and 100 cells/ml of *L. monocytogenes* the regulatory limits were predicted to be met in only 38%, 29% and 23% of cases respectively.

Table 4: Estimates of *L. monocytogenes* concentration in raw milk Cheddar cheese made from milk with different initial contamination levels

	Initial contamination in raw milk				
	0.001 cell/ml	0.1 cell/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	1.87×10^{-1}	1.74×10^{1}	1.81×10^{2}	1.73×10^{3}	1.82×10^{4}
Mean	1.41×10^{0}	1.39×10^{2}	1.39×10^{3}	1.39×10^{4}	1.40×10^{5}
Maximum	1.09×10^{1}	8.98×10^{2}	8.05×10^{3}	8.67×10^{4}	9.45×10^{5}
5^{th} percentile	3.32×10^{-1}	3.30×10^{1}	3.30×10^{2}	3.27×10^{3}	3.32×10^{4}
95^{th} percentile	3.69×10^{0}	3.65×10^{2}	3.61×10^{3}	3.65×10^{4}	3.64×10^{5}
End of 6 months maturation concentration in cheese (cells/g)					
Minimum	0	0	0	0	0
Mean	1.50×10^{-2}	1.47×10^{0}	1.52×10^{1}	1.52×10^{2}	1.50×10^{3}
Maximum	2.00×10^{0}	1.77×10^{2}	1.84×10^{3}	2.11×10^{4}	2.68×10^{5}
5^{th} percentile	2.54×10^{-14}	9.06×10^{-13}	2.40×10^{-11}	1.72×10^{-10}	2.00×10^{-9}
95^{th} percentile	7.42×10^{-2}	7.39×10^{0}	7.45×10^{1}	7.48×10^{2}	7.43×10^{3}

3.4 Sensitivity analysis

A sensitivity analysis was performed on the model for raw milk Cheddar cheese to determine the variables with the greatest influence on the final concentration of pathogens in the cheese. The resulting tornado plots (Figures 10 – 12) show the regression sensitivity results for *E. coli, L. monocytogenes* and *S. aureus,* respectively. For some model inputs a distribution of values was not available and were therefore modelled using fixed values (for example the initial decrease in pH during manufacture). The sensitivity analyses only consider those inputs described using distributions. The relative importance of each input distribution to the final pathogen concentration can be gauged by the magnitude of the standardised b coefficient. A large positive value suggests a strong relationship, while a value close to zero indicates a weak relationship. A general rule-of-thumb is that values greater than 0.5 are of practical significance and that a linear relationship exists between the two variables.

The tornado plots for each of the three pathogens follow a similar pattern (Figure 10-12) with the decimal reduction time having the largest impact on the final concentration in the cheese. This is most strongly observed in the case of *L. monocytogenes* where the coefficient is +0.899. For *E. coli* and *S. aureus* the relationship is weaker at 0.357 and 0.484, respectively.

No processing factors were found to have strong impact on the final pathogen concentration. The factor identified as the second ranked factor for two pathogens was the curd press time, however the relationship was very weak with values of less than 0.2.

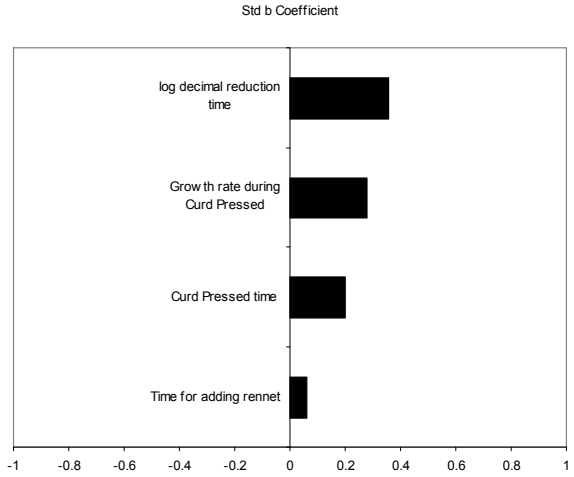

Figure 10: Regression sensitivity analysis results for *E. coli* in raw milk Cheddar cheese matured 26 weeks

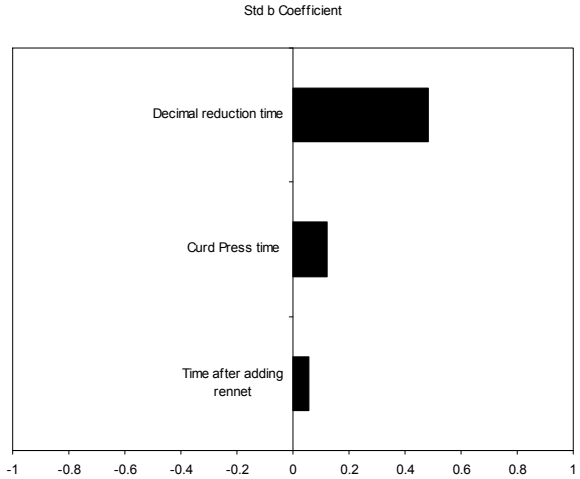

Figure 11: Regression sensitivity analysis results for *S. aureus* in raw milk Cheddar cheese matured 26 weeks

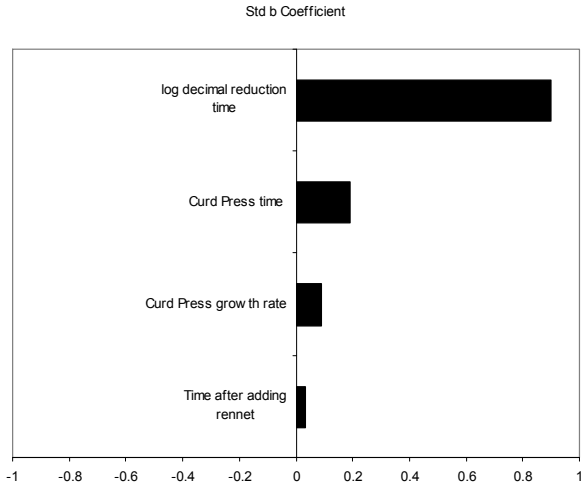

Figure 12: Regression sensitivity analysis results for *L. monocytogenes* in raw milk Cheddar cheese matured 26 weeks

3.5 Summary of modelled results on effect of manufacture

Results from the quantitative model estimated that the net change in *E. coli* from the initial level in the raw milk to the final ripened cheese was an increase of 1 log. For a cheese to meet current requirements in the Code it is estimated that the initial concentration of *E. coli* in the raw milk should be no greater than 0.1 cfu/ml.

The predicted inactivation of *S. aureus* during the ripening of Cheddar cheese was greater than 7 log, resulted in low estimated levels of *S. aureus* in the final cheese product. However, during the initial stages of cheese production there was an estimated 3 log increase in *S. aureus*. If 100 cfu/ml *S. aureus* were present in the raw milk, levels may potentially reach 10^5 cfu/g following the fermentation stage and prior to ripening. At this level, enterotoxin production was of concern.

Experimental results for *L. monocytogenes* were highly variable, due largely to variations in inactivation between strains during ripening. During the initial manufacture of Cheddar cheese, the estimated growth of *L. monocytogenes* was 3 log, followed by a 5 log reduction during ripening. To consistently produce cheese that would meet the requirements in the Code it was estimated that the initial level in raw milk would need to be less than 0.001 cfu/ml.

A summary of the estimated impact on the concentration of specific microorganisms during the production of Cheddar cheese is summarised in Table 5.

Table 5: Predicted changes in concentration of microorganisms during raw milk Cheddar cheese production (using a Log 10 scale)

	E. coli	***S. aureus***	***L. monocytogenes***
End of fermentation	+4.13	+2.86	+3.02
End of maturation	-3.15	-7.55	-5.36
Net change	+0.98	-4.69	-2.34

2.6 Consumption of Cheddar cheeses in Australia

Data from the 1995 Australian National Nutrition Survey[43] (NNS) gives an indication of the percentage of the population who consume various types of cheese and the amount they consume.

Hard cheeses, such as Cheddar cheese are the major type of cheese consumed in Australia. Table 6 depicts data from the NNS on the Australian average daily consumption of Cheddar cheese by gender and age. Data indicates 26.5% of those surveyed consumed Cheddar cheese with the average amount being 35g. Cheddar cheese was consumed by all age groups.

[43] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24 hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

It cannot be assumed that this same proportion of the population would also consume raw milk cheese. However it is likely that those who will consume raw milk cheese, will not increase their cheese consumption, rather they will substitute consumption of pasteurised cheese with raw milk cheese.

Table 6: Australian average daily consumption Cheddar cheese by gender and age (Australian Government Department of Health and Family Services, 1997)

Gender	Age	No. of respondents	No. of consumers (% of respondents)		Average amount of cheese consumed (g/day)
Male	2 - 3	170	37	(21.8)	45
Male	4 - 7	416	97	(23.3)	37
Male	8 - 11	385	77	(20.0)	23
Male	12 - 15	349	89	(25.5)	21
Male	16 - 18	215	72	(33.5)	39
Male	19 - 24	485	143	(29.5)	50
Male	25 - 44	2140	655	(30.6)	40
Male	45 - 64	1554	421	(27.1)	35
Male	65+	902	233	(25.8)	23
Female	2 - 3	213	50	(23.5)	21
Female	4 - 7	383	73	(19.1)	23
Female	8 - 11	354	96	(27.1)	37
Female	12 - 15	304	71	(23.4)	45
Female	16 - 18	218	61	(28.0)	39
Female	19 - 24	575	151	(26.3)	50
Female	25 - 44	2385	656	(27.5)	41
Female	45 - 64	1752	448	(25.6)	35
Female	65+	1058	243	(23.0)	23

4 Risk characterisation

In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles (Appendix 1).

The qualitative framework considers the characterisation of identified hazards (hazard identification and characterisation combined) and an assessment of the likely exposure to these hazards (exposure assessment) which when combined provides a characterisation of the risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of disease. The exposure module characterises exposure to the hazard based on the likely level of the hazard in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of the hazard on a "per serve" basis[44]. Essentially, the matrix categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

The model was employed to characterise the risk from raw milk Cheddar cheese.

[44] "per serve" is defined as the amount of product consumed per eating occasion.

Risk categories for hazards in raw milk Cheddar cheese were assigned as follows:

Hazard	Infective dose	Consequence of exposure	Severity of hazard
E. coli (EHEC)	<10	Serious	High
S. aureus	>1,000	Mild	Negligible
L. monocytogenes	>1,000/10-100[#]	Moderate/Severe[#]	Negligible/Moderate[#]

susceptible populations

Exposure categories for raw milk Cheddar cheese:

Pathogen	Raw product contamination	Effect of processing	Exposure
E. coli (EHEC)	Infrequent (1%)	10 fold increase	Moderate
S. aureus	Sometimes (10%)	50% reduction	Low
L. monocytogenes	Infrequent (1%)	50% reduction	Very low

Risk characterisation for raw milk Cheddar cheese:

Pathogen	Hazard	Exposure	Risk Characterisation
E. coli (EHEC)	High	Moderate	High
S. aureus	Negligible	Low	Very low
L. monocytogenes	Negligible/Moderate[#]	Very low	Negligible/Low[#]

susceptible populations

5 Conclusions

During the production of raw milk Cheddar cheese it was predicted that there would be an overall increase of 0.98 logs in the concentration of *E. coli* from the initial levels in the raw milk to those present in the final cheese. The overall concentration of *L. monocytogenes* in the final cheese would decrease by 2 logs in concentration compared with those observed initially in the raw milk. However, inactivation and survival was very variable depending upon the different strains.

The probabilistic model predicted high inactivation of *S. aureus* during the ripening of Cheddar cheese (>7 log), however during the initial stages of cheese production there was an estimated 3 log increase (*i.e.* if 100 cfu/ml *S. aureus* were present in the raw milk, this resulted in levels reaching 10^5 cfu/g in the cheese prior to ripening).

Challenge studies have reported variable results for reductions in *E. coli* during ripening and maturation of Cheddar cheese. Reitsma and Henning (1996) examined the survival of *E. coli* O157:H7 during manufacture and ripening of Cheddar cheese, and reported a 2.8 - 5.8 log reduction after 22.5 weeks at 6 - 7°C. Teo *et al*. (2000) found *E. coli* O157:H7 exhibited a less than 1 log reduction during 60 days of aging, and only a 1 - 2 log reduction by 90 days (Teo *et al*., 2000). More recently, Schlesser *et al*. (2006) examined the survival of *E. coli* O157:H7 and report a 1 - 2 log reduction in Cheddar cheese.

Ryser and Marth (1987b) demonstrated that *L. monocytogenes* can persist for up to 434 days at 6°C post-processing in artificially contaminated Cheddar cheese. In addition, Ryser and Marth (1987b) and Yousef and Marth (1990) found that numbers of *L. monocytogenes* gradually decreased during ripening/maturation in Cheddar and Colby cheeses and that the decline in population is strongly influenced by the moisture content and pH. However more

recent research has shown that *S. typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* can survive well beyond the 60-day holding period in Cheddar cheese prepared from pasteurised milk (Reitsma and Henning, 1996; Ryser and Marth, 1999).

The process of manufacturing raw milk Cheddar cheese has been assessed with respect to its affect on selected pathogens as follows:

Pathogen	Risk associated with raw milk Cheddar cheese
E. coli (EHEC)	**High** risk as the organism survives the cheesemaking process and cheese maturation.
S. aureus	Risk from staphylococcal enterotoxin is considered **very low**. Conditional on good control over animal health and raw milk handling. The organism doesn't survive ripening/ maturation.
L. monocytogenes	**Negligible** risk (general population) and **low** risk (susceptible population groups) as the organism survives the cheesemaking process, however there is a large variability between strains on survival.*.

* For raw milk cheese manufactured from sheep milk the risk is very low for general population and moderate for susceptible population groups

There is considered to be little difference in the public health and safety risk from *E. coli* (EHEC) and *S. aureus* in raw milk Cheddar cheeses made from either cow, goat or sheep milk. However, *L. monocytogenes* presents a greater risk in raw milk Cheddar cheese when produced from raw sheep milk compared to raw cow or raw goat milk, due to its higher reported prevalence in sheep milk.

Cheddar cheese is the most common type of cheese consumed in Australia. It is possible that if raw milk Cheddar cheese was available, it may be consumed by all segments of the population.

The microbiological safety of raw milk Cheddar cheeses appears to be largely dependent upon the microbiological quality of the raw milk and rapid acidification (*i.e.* pH <5.5 after 3 - 6 hours).

Quantitative modelling has shown that in order to produce raw milk Cheddar cheese that would meet current microbiological limits in the Code, the initial concentration of *E. coli*, and *L. monocytogenes* in the raw milk would need to be less than 0.01 and10^{-3} cfu/ml, respectively. To produce Cheddar cheese unlikely to have a level of *S. aureus* cells that could generate sufficient staphylococcal toxin to cause illness (*i.e.* $<10^5$ cfu/g), the initial concentration in the milk would need to be less than 100 cfu/ml.

The extent that these findings could be applied across the breadth of Cheddar cheese varieties is variable. It could be assumed that the same level of risk would apply to all raw milk Cheddar cheeses whose manufacturing specifications lie within the range of those of the modelled raw milk Cheddar cheese. However, the findings of the modelled raw milk Cheddar cheese assessed cannot be applied to other hard cheeses based on moisture (*i.e.* 37 - 42% moisture) as the modelled cheeses do not represent all types of hard cheeses in respect to physicochemical characteristics and manufacturing protocols.

Appendix 11: Risk assessment – raw milk blue cheese

1 Introduction

Blue cheese is an internally mould ripened cheese characterised by a network of blue and green veins running continually throughout the cheese due to the growth of *Penicillium roqueforti*. Many countries have developed their own types of Blue cheese, each with different characterisitics and manufacturing methods. Some well-known examples include: Gorgonzola, Danablue, Stilton and Roquefort, all of which have been granted Protected Designation of Origin/Protected Geographical Indication (PDO/PGI) status. Blue cheeses can be made with cow, sheep or goat milk, or a mixture thereof.

During cheese manufacture, the curds are generally cooked at low temperatures before transfer to drainers or moulds for separation of whey from the curds. Blue cheeses can be either dry or brine salted, and are ripened in aerobic conditions to favour mould growth. Ripening temperatures typically range from 8 - 15^{o}C depending on the variety. The cheese is punctured (needling) to allow oxygen to enter the interior of the cheese and CO_2 produced by the mould to escape. Considerable structural differences exist within these cheeses which influences the level and distribution of O_2 and CO_2. The minimum pH of blue cheeses ranges from approximately 4.6 – 4.7 in Danablue and Stilton to 5.15 -5.30 in Gorgonzola and Cabrales. When mature, cheeses can have pH up to 6.0 – 6.5. .

Classification of blue cheeses differs depending on the classification system used (Scott (1986) Ottogalli (1998, 2000a, 2000b, 2001) and Fox *et al.* (2000). Based solely on moisture content, the classification system of Scott (1986) categorises these cheeses as "semi-hard" (44 - 55% moisture). Some blue cheeses have moisture contents greater than 55% and would be classified as "soft", *i.e*. Kopanisti cheese (69.4% moisture). Characteristics of various blue cheeses are outlined in Appendix 3 and vary considerably.

This risk assessment examines the fate of *L. monocytogenes,* during the manufacture of a generic raw milk blue cheese based on a probabilistic model developed by the University of Tasmania and adapted by FSANZ. There was insufficient data on pathogen reduction for *E. coli* and *S. aureus* during the ripening phase of blue cheese for modelling of these organisms to be undertaken.

The manufacturing parameters and physicochemical properties for the modelled raw milk blue cheese are based on experimental data and do not necessarily reflect commercial manufacturing practices or a particular variety of blue cheese. The modelled raw milk blue cheese manufacturing parameters and physicochemical characteristics are described in Figure 1.

A qualitative framework was subsequently used to rate the risk to public health and safety from *L. monocytogenes* from the consumption of the modelled raw milk blue cheese made from cow, goat or sheep milk.

2 Hazard identification and hazard characterisation

In evaluating the safety of blue cheese, only *L. monocytogenes* was considered in this assessment. A detailed characterisation of potential hazards is attached as Appendix 14.

3 Exposure assessment

The production steps for blue cheese manufacture were adapted from Papageorgiou and Marth (1989) and Schaffer *et al*. (1995). A summarised conceptual model of the production flow is illustrated in Figure 1.

A summary of input variables that are represented by distribution functions is shown (Table 1). It has been assumed that the volume of milk used to make 1 kg of blue cheese is approximately 10 litres.

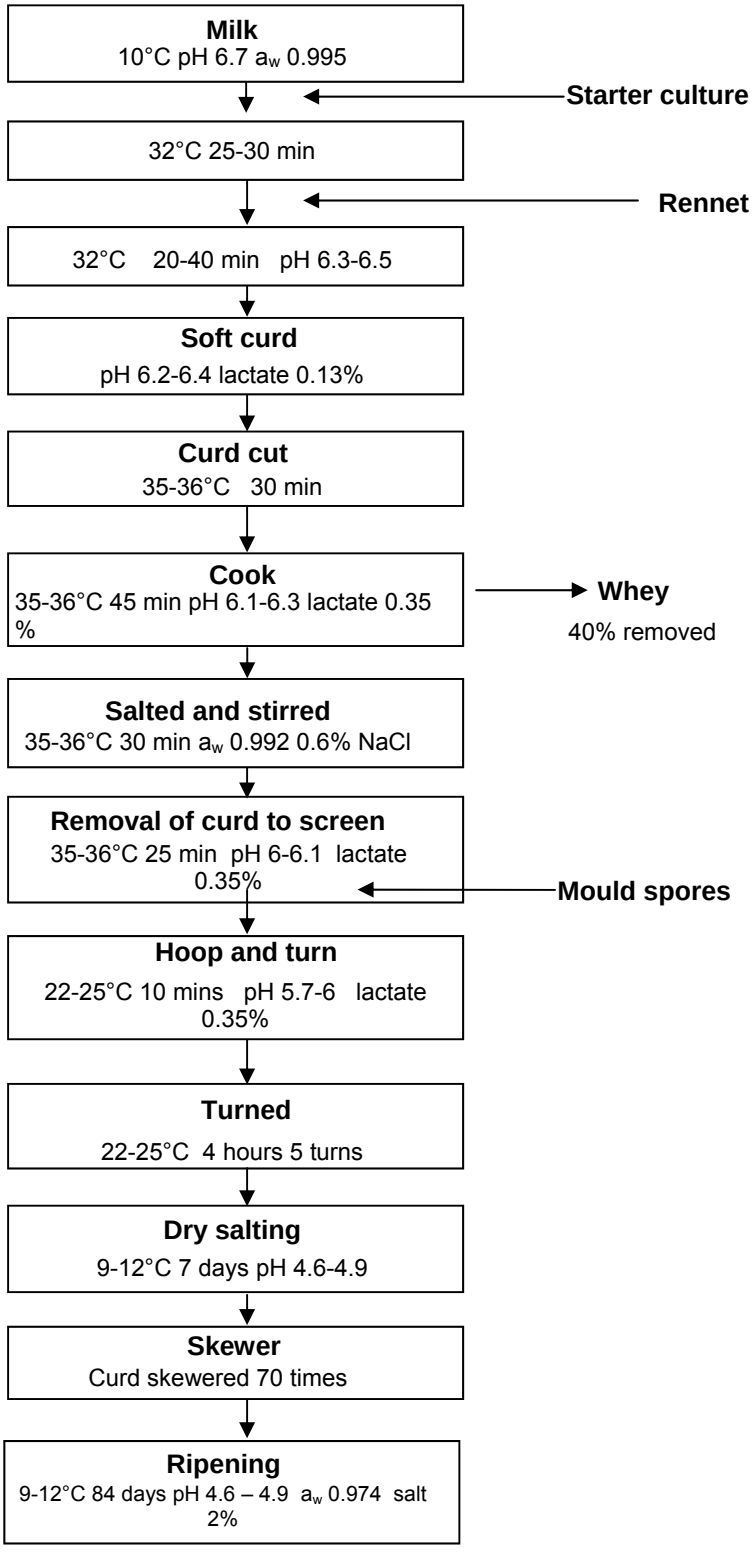

Figure 1: Conceptual model of blue cheese production

Table 2: Input distribution functions used in the models for blue cheese

Cheese type	Step	Input Parameter	Distribution
Blue	Rennet added	Temperature, ºC	Uniform (31, 32)
		Time, minutes	Uniform (20, 60)
	Curd Cut	pH	Uniform (6.3, 6.5)
		Temperature, ºC	Uniform (31, 32)
	Cook	Temperature, ºC	Uniform (35, 36)
		pH	Uniform (6.1, 6.3)
	Salted and Stirred	pH	Uniform (6.1, 6.2)
	Curd to Screen	Temperature	Uniform (35, 36)
		pH	Uniform (6.0, 6.1)
	Hoop and Turn	Temperature, ºC	Uniform (22, 25)
		pH	Uniform (5.7, 6.0)
	Turning	Temperature ºC	Uniform (22, 25)
	Dry Salting	Temperature, ºC	Uniform (9, 12)
		pH	Uniform (4.6, 4.9)
	Ripening	Temperature, ºC	Uniform (9, 12)
		pH	Uniform (4.6, 4.9)

3.1 Initial phase of manufacture

During the initial phase of manufacture of blue cheese, bacterial pathogens concentrate within the curd matrix as it forms (Papageorgiou and Marth, 1989b). Some are excluded in the whey; however this is only a small fraction of the total concentration.

The results of Papageorgiou and Marth (1989b) suggest that acid production and the resultant decline in pH during the initial phase of cheese production is the major factor inhibiting the growth *L. monocytogenes*.

3.2 Ripening

Data on pathogen reduction during the maturation phase of blue cheese was not available for *E. coli* or *S. aureus*, however sufficient data was available to model the effect of ripening on *Listeria monocytogenes*. The Whiting *et al* (1996) model was not used to model the inactivation in blue cheese due to a lack of validating data.

In blue cheese the inactivation of *L. monocytogenes* was more pronounced in the first 40 days of ripening, however, once the pH of the cheese rose above pH 5, there was no further inactivation (Papageorgiou and Marth, 1989b). Data from Schaffer *et al*. (1995) and Papageorgiou and Marth (1989b) were used to fit a linear inactivation model to determine decimal reduction times to predict the decline in *L. monocytogenes* concentration during ripening. Using the data for the strains Scott A and California the decimal reduction times were estimated using a Normal distribution of $\log_{10} D$ (days) with a mean of 1.16 and standard deviation of 0.17.

In order to extend the application of the probabilistic model, the time to reach a pH of 5 during ripening was predicted, rather than using a fixed time of 40 days as used in the University of Tasmania approach. This was achieved by using an adapted form of the Baranyi equation to define the dynamic changes in pH throughout the ripening of the model blue cheese of Papageorgiou and Marth (1989b). The result was a Normal distribution describing the time at which the pH reached 5, with a mean of 37.6 days and standard deviation of 4.8 days.

3.3 Probabilistic model estimates

Blue cheese made from raw milk contaminated with *L. monocytogenes* at a concentration of 10^{-3} cells/ml was predicted to fail the regulatory limits in 55% of cases (Figure 2). The average concentration in matured blue-cheese was 2.75 x 10^{-2} cells/g, with a maximum value of 5.71 cell/g and minimum 9.51 x 10^{-6} cells/g. All other initial contamination levels resulted in concentrations in matured blue cheese that exceeded the limit in more than 85% of iterations. A summary of the estimated concentration of *L. monocytogenes* in raw milk blue cheese from different initial contaminations of raw milk is shown in Table 2.

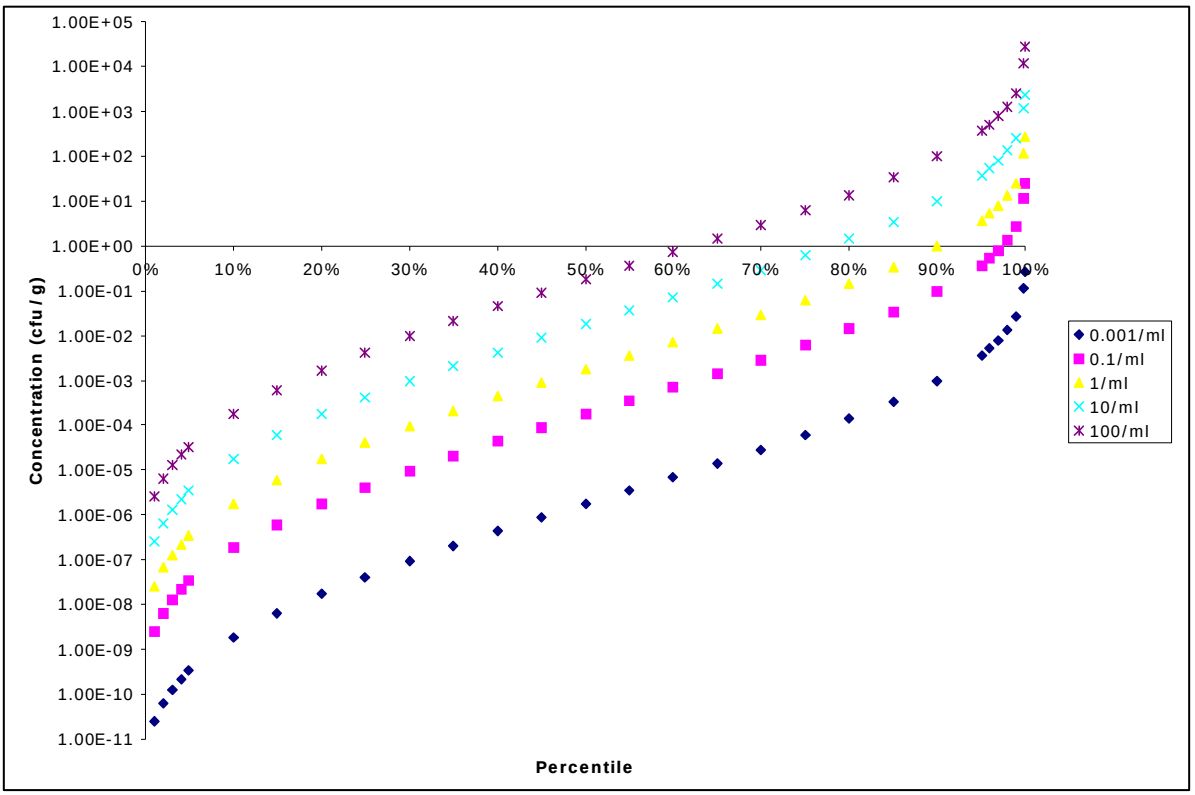

Figure 2: Estimated concentration of *L. monocytogenes* in raw milk blue cheese matured for 84 days with different initial contamination in milk

Table 2: Estimates of *L. monocytogenes* concentration in raw milk blue cheese made from milk with different initial contamination levels

	Initial contamination in raw milk				
	0.001 cell/ml	0.1 cell/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	6.82×10^{0}	7.81×10^{2}	8.33×10^{3}	6.18×10^{4}	8.37×10^{5}
Mean	5.76×10^{1}	5.73×10^{3}	5.85×10^{4}	5.94×10^{5}	5.75×10^{6}
Maximum	8.48×10^{2}	6.14×10^{4}	9.68×10^{5}	3.99×10^{7}	6.96×10^{7}
5th percentile	1.90×10^{1}	1.81×10^{3}	1.84×10^{4}	1.90×10^{5}	1.88×10^{6}
95th percentile	1.37×10^{2}	1.39×10^{4}	1.43×10^{5}	1.40×10^{6}	1.35×10^{7}
End of 90-day maturation concentration in cheese (cells/g)					
Minimum	3.03×10^{-10}	1.93×10^{-8}	1.56×10^{-11}	2.33×10^{-7}	3.62×10^{-8}
Mean	6.25×10^{-1}	6.31×10^{1}	6.15×10^{2}	6.55×10^{3}	6.29×10^{4}
Maximum	3.83×10^{1}	2.97×10^{3}	3.08×10^{4}	1.72×10^{6}	5.24×10^{6}
5th percentile	3.61×10^{-4}	3.33×10^{-2}	3.17×10^{-1}	3.81×10^{0}	3.61×10^{1}
95th percentile	2.80×10^{0}	2.72×10^{2}	2.70×10^{3}	2.72×10^{4}	2.92×10^{5}

3.4 Sensitivity analysis

As for Cheddar cheese, the decimal reduction time during maturation of raw milk blue cheese had the greatest impact on the final concentration of *L. monocytogenes*. (Figure 3). The curd pH (with resulting faster growth rates) and the time during maturation when the pH reached 5 had little impact on the final concentration of *L. monocytogenes*.

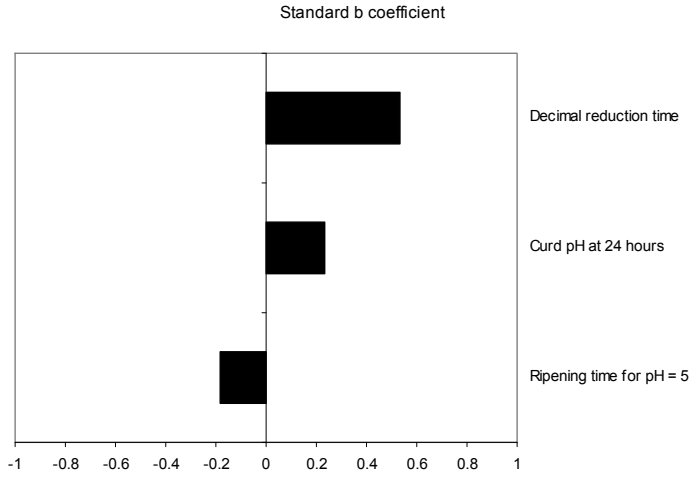

Figure 3: Regression sensitivity analysis results for *L. monocytogenes* in raw milk blue cheese matured for 90 days

3.5 Summary of modelled results on effect of manufacture

The model estimated that the mean reduction during a 90-day maturation period was 2.8 log. Taking into account the predicted 4.7 log growth during initial stages of production (first 24 hours), the net effect was an increase of approximately 1.8 log from the original contamination level in the milk. An increase in *L. monocytogenes* during the initial manufacture of blue cheese has been observed by Papageorgiou and Marth (1989b).

A summary of the estimated impact on the concentration of specific microorganisms during the production of raw milk blue cheese is summarised in Table 3.

Table 3: Predicted changes in concentration of microorganisms during raw milk blue cheese production (using a Log 10 scale)

	E. coli	***S. aureus***	***L. monocytogenes***
First 24 hours	n/a	n/a	+4.67
Maturation	n/a	n/a	-2.81
Net Change	n/a	n/a	+1.86

3.6 Consumption of blue cheeses in Australia

Data from the 1995 Australian National Nutrition Survey[45] (NNS) gives an indication of the percentage of the population who consume various types of cheese and the amount they consume.

Blue cheese is not a commonly consumed food in Australia, with only approximately 0.5 % of NNS respondents consuming blue vein type cheese. The average amount consumed was 36.6 g (Table 4).

It cannot be assumed that this same proportion of the population would also consume raw milk cheese. However it is likely that those who will consume raw milk cheese, will not increase their cheese consumption, rather they will substitute consumption of pasteurised cheese with raw milk cheese. Therefore consumption of raw milk blue cheese, if available, is likely to be extremely low.

Table 4: Australian average daily consumption of blue cheese by gender and age (Australian Government Department of Health and Family Services, 1997)

Age (years)	No. surveyed	No. consuming blue cheese (% of no. surveyed)	Mean consumer intake of blue cheese (g/day)
2 - 4	583	0 (0%)	0
5 - 12	1,496	0 (0%)	0
13 - 18	928	1 (0.1%)	71.5
19 - 64	8,891	49 (0.6%)	20.8
65+	1,960	15 (0.8%)	17.6
TOTAL	**13,858**	**65 (0.5%)**	**36.6**

4 Risk characterisation

In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles (Appendix 1).

The qualitative framework considers the characterisation of identified hazards (hazard identification and characterisation combined) and an assessment of the likely exposure to these hazards (exposure assessment) which when combined provides a characterisation of the risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of disease. The exposure module characterises exposure to the hazard based on the likely level of the hazard in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of the hazard on a

[45] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24 hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

"per serve" basis[46]. Essentially the matrix categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

The model was employed to characterise the risk from raw milk blue cheese.

Risk categories for hazards in raw milk blue cheese were assigned as follows:

Pathogen	Infective dose	Consequence of exposure	Severity of hazard
L. monocytogenes	>1,000/10-100#	Moderate/Severe#	Negligible/Moderate#

susceptible populations

Exposure categories for raw milk blue cheese:

Pathogen	Raw product contamination	Effect of processing	Exposure
L. monocytogenes	Infrequent (1%)	10 fold increase	Moderate

Risk characterisation for raw milk blue cheese:

Pathogen	Hazard charcterisation	Exposure assessment	Risk Characterisation
L. monocytogenes	Negligible/Moderate#	Moderate	Low/High#

susceptible populations

5 Conclusions

During the production of the modelled raw milk blue cheese it was estimated that there would be an overall increase of approximately +1.86 log in the concentration of *L. monocytogenes* from the initial levels in the raw milk to that present in the final cheese. The relatively low inactivation predicted during maturation is insufficient to reduce levels from those present in the raw milk.

Ryser and Marth (1987b) found numbers of *L. monocytogenes* to decrease abruptly during early ripening of blue cheese; however as the pH approaches neutral levels during ripening, growth would be permitted. Papageorgiou and Marth (1989b) examined the fate of *L. monocytogenes* and found it failed to grow and decreased in number during 56 days of storage and suggested that *P. roqueforti* may produce bacteriocins against *L. monocytogenes*.

There was insufficient data to model *E. coli* and *S. aureus* in raw milk blue cheese. However, de Boer and Kuik (1987) examined 256 samples of blue vein cheeses (Roquefort, Danablu, and Gorgonzola) and found that *S. aureus* was always present at numbers less than 100 cfu/g (De Boer and Kirk, 1987). Tatini *et al*. (1973) studied the production of enterotoxin A in blue cheese, and could not detect enterotoxin in any lots, even when large inocula ($>10^6$ cfu/ml) were used and *S. aureus* populations reached 10^7 cfu/g of cheese, or when a complete starter failure was induced by bacteriophage action (Tatini *et al*., 1973).

The existing data suggest that cheeses ripened with internal mould activity present a very hostile environment for *S. aureus*. This may be due to the combined inhibitory effect of *Penicillium* spp. and starter bacteria (Meyrand, 1998; Tatini *et al*., 1973).

[46] "per serve" is defined as the amount of product consumed per eating occasion.

Based on this assessment, the process of manufacturing raw milk blue cheese has been assessed to affect selected pathogens as follows:

Pathogen	Risk associated with raw milk blue cheese
L. monocytogenes	**Low** risk (general population) and **high** risk (susceptible population groups) as the organism increases in numbers during the cheesemaking process.

It is considered that there is little difference in the public health and safety risk from *L. monocytogenes* in raw milk blue cheeses made from either cow, goat or sheep milk.

Consumption of raw milk blue cheese, if available, is likely to be extremely low, however changing eating patterns may see this increase.

The microbiological safety of raw milk blue cheese appears to be largely dependent upon the microbiological quality of the raw milk and rapid acidification (*i.e.* <5.5 within 3 - 6 hours).

Quantitative modelling has shown that in order to produce raw milk blue cheese that would meet current microbiological limits in the Code, the initial concentration of *L. monocytogenes* in the raw milk would need to be less than 10^{-5} cfu/ml.

There is significant variability between physicochemical characteristics and cheese manufacturing conditions of different types of blue cheeses and as such the safety assessment of *L. monocytogenes* can only be applied to the modelled raw milk blue cheese (*i.e.* the safety of *L. monocytogenes* cannot be ascertained for other blue cheeses).

To highlight this point, during the assessment of Application A499 – To permit the sale of Roquefort cheese[47], the scientific evaluation was able to determine the risk from a number of pathogens from challenge studies and detailed manufacturing protocols specific to Roquefort cheese. *L. monocytogenes* was assessed as being a very low to negligible risk for all populations in Roquefort cheese, whereas for the modelled raw milk blue cheese, a low risk rating was assessed for the general population and a high risk rating for susceptible populations.

Differences in the risk of *L. monocytogenes* between the previously assessed Roquefort cheese and the modelled raw milk blue cheese can be attributed to the different physicochemical characteristics of each cheese and the inhibitory effect these have on the ability of *L. monocytogenes* to grow and/or survive during manufacture and ripening.

In both the modelled blue cheese and assessed Roquefort cheese, *L. monocytogenes* grew during the initial stage of manufacture. The extent of die-off which occurs during subsequent stages of manufacture and during the ripening period is dependent upon factors such as pH, water activity, salt content and the time and temperature of storage/ripening.

The modelled blue cheese has a lower salt content (2% compared with 3%), higher water activity (0.97 compared to 0.92) and lower pH at 90 days (5.2 compared to 5 - 6.0) than Roquefort cheese. This environment is more favourable for the survival of *L. monocytogenes* compared to that of Roquefort cheese, resulting in less death of *L. monocytogenes* during ripening and the consequent greater survival.

[47] Application A499 – To permit the sale of Roquefort Cheese http://www.foodstandards.gov.au/_srcfiles/A499_Roquefort_FAR_FINALv2.doc.

The extent that the findings of the modelled raw milk blue cheese can be applied across the breadth of blue cheese varieties is uncertain. It could be assumed that the same level of risk would apply to those raw milk blue cheeses whose manufacturing specifications lie within the range of those used for the modelled cheese. However, the findings of the modelled raw milk blue cheese assessed cannot be applied to other semi-soft cheeses based on moisture (*i.e.* 43 - 55%) as the physicochemical characteristics and manufacturing protocols of the modelled blue cheese do not represent all types of semi-soft cheeses.

Appendix 12: Risk assessment – raw milk Feta cheese

1 Introduction

Feta cheese is characterised as a cheese ripened under brine. Coagulation of Feta cheese is achieved using rennet and acidification is achieved using either thermophilic or mesophilic lactic bacteria as a starter culture. The coagulum for Feta cheese is cut and the soft curds are ladled directly into moulds and left to drain until cohesion occurs. When the curd mass is firm it is removed from the moulds and cut into blocks, salted and transferred to a brine solution for ripening, and generally stored at 2 - 4°C for at least 2 months.

Feta cheeses are classified differently by each of the classification systems presented by Scott (1986), Ottogalli (1998, 2000a, 2000b, 2001) and Fox *et al.* (2000). However, based solely on moisture content, Scott (1986) categorises these cheeses as "soft". The characteristics of typical Feta cheese are outlined in Appendix 3.

This risk assessment examined the fate of Enterohaemorragic *Escherichia coli* , *Staphylococcusaureus* and *Listeria monocytogenes* during the manufacture of a raw milk Feta cheese using a probabilistic model developed by the University of Tasmania and adapted by FSANZ.

The manufacturing parameters and physicochemical properties for the modelled raw milk Feta cheese are based on experimental data and do not necessarily reflect commercial manufacturing practices. The modelled raw milk Feta cheese manufacturing parameters and physicochemical characteristics are described in Figure 1.

A qualitative framework was subsequently used to rate the risk to public health and safety from the consumption of raw milk Feta cheese made from cow, goat or sheep milk, containing these microbiological hazards.

2 Hazard identification and hazard characterisation

In evaluation the safety of raw milk Feta cheese the following pathogens: *E. coli*, *L. monocytogenes*, and *S. aureus* were considered in this assessment. A detailed characterisation of potential hazards is attached as Appendix 14.

3 Exposure assessment

To simulate the fate of pathogens during the production of raw milk Feta cheese a model was developed from published descriptions of Feta cheese production (Govaris, 2002; Papageorgiou and Marth, 1989a; Ramsaran, 1998). A conceptual flow diagram of the production process is given in Figure 1. Listed in Table 1 are the steps and variables involved in Feta manufacture. There are several variations in production steps for Feta cheese, primarily the salt concentrations and time/temperature combinations. There are two distinct salting stages. Papageorgiou and Marth (1989a)used a high salt brine and a low salt brine in two stages, whereas Govaris *et al.* (2002) and Ramsaran *et al*. (1998) had a dry salting stage followed by brining of the cheese in a low salt brine.

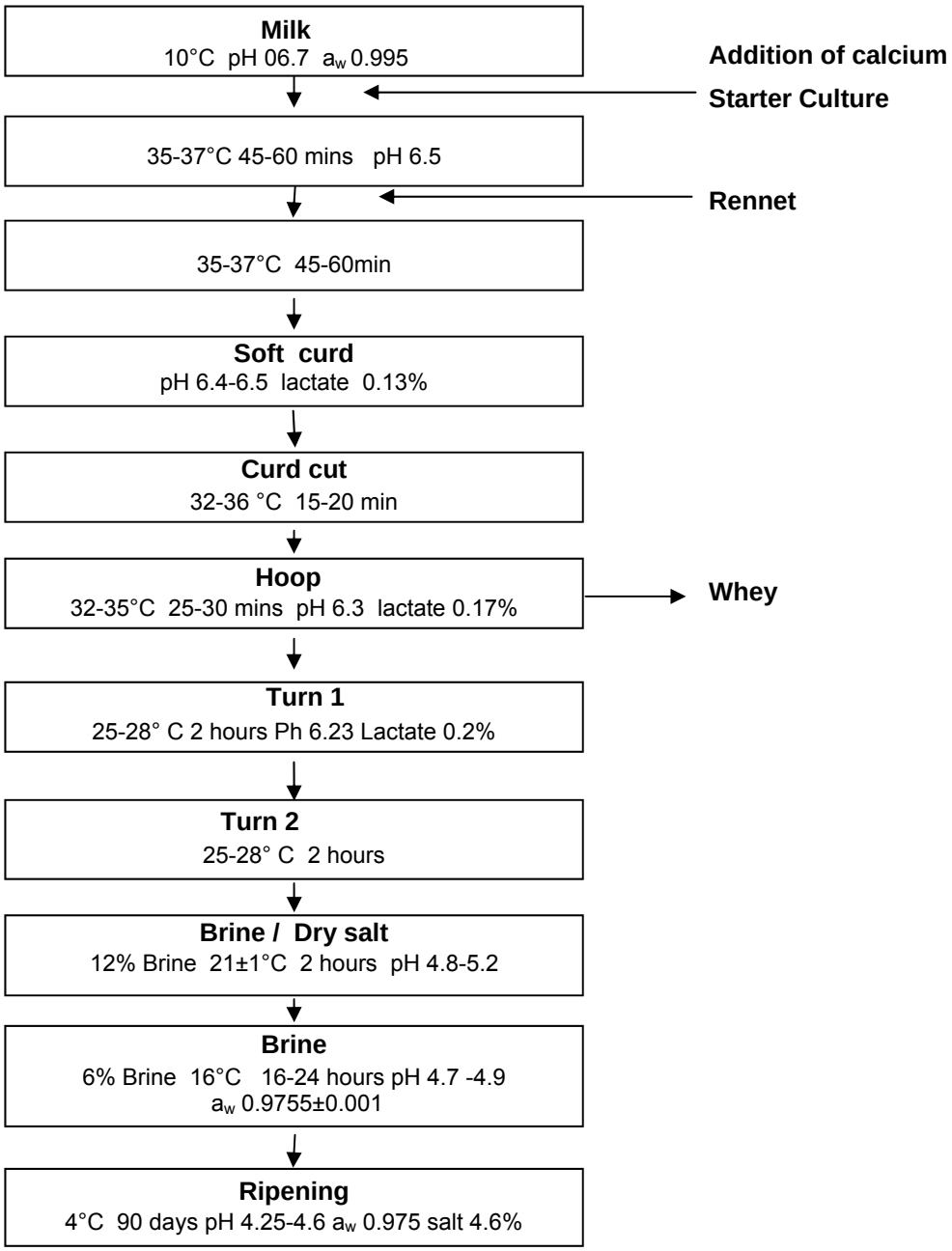

Figure 1: Conceptual flow diagram for the production of raw milk Feta cheese

Table 1: Processing steps involved in the production of raw milk Feta cheese and the distribution functions for variables in each step

Step	Parameter	Distribution function ‡
Add starter	Temperature (ºC) Time (min)	Uniform (35, 37) Uniform (45, 60)
Add Calcium Chloride		
Add rennet	Temperature (ºC) Time (min)	Uniform (35, 37) Uniform (45, 60)
Cut curd	Temperature (ºC) Time (min)	Uniform (32, 36) Uniform (15, 20)
Hoop curd	Temperature (ºC) Time (min)	Uniform (32, 35) Uniform (25, 30)
Whey Drained	% cells lost	Normal (3.21, 2.18) Truncated at 0
1st turn	Temperature (ºC) Time (hours)	Uniform (25, 28) 2
2nd turn	Temperature (ºC) Time (hours)	Uniform (24, 26) 2
Brine high salt cheese †	Temperature (ºC) pH water activity/salt Time (hours)	Normal (22, 1) Uniform (4.8, 5.2) Normal (0.9878, 0.0012)/Normal (2.2, 0.18) 2
Brine low salt cheese †	Time (hours) pH water activity/salt	Discrete (16, 24) Uniform (4.7, 4.9) Normal (0.9755, 0.001)/Normal (4.573, 0.22)
Ripening	Time (days) Temperature (ºC)	90 4

‡ Distribution functions are derived (Govaris, 2002; Papageorgiou and Marth, 1989a; Ramsaran, 1998).
† Salt concentration is considered part of the salt in water phase of the cheese

The volume of milk needed to make 1kg of raw milk Feta varies according to the type of milk used for production, sheep milk being traditionally used in Greece. The higher protein and solid content of sheep and goat milk means that approximately 6 litres of milk is required, whereas 9 litres of cow milk is required (Govaris, 2002).

3.1 Initial phase of manufacture

Growth of starter cultures and non-starter bacteria during cheese production has an impact on the growth potential of pathogens that may be present in the milk. Total counts of lactic acid bacteria and non-starter lactic acid bacteria may reach 10^8-10^9/g in cheese by the start of maturation (Manolopoulou *et al*., 2003). Erkmen (1995) observed a 4 log increase in the concentration of lactic acid bacteria during Feta cheese manufacture. The effects of competition between pathogenic bacteria and starter culture bacteria have not been modelled; however the impact of changes in the pH caused by lactic acid bacteria is modelled.

A proportion of bacterial cells present in the cheese curd are lost into the whey when it is drained. Papageorgiou and Marth (1989a) determined that 3.2% of *L. monocytogenes* cells were lost to the whey during draining. This was similar to the 1 - 3% loss of *L. monocytogenes* to whey observed during the production of Colby cheese (Yousef and Marth, 1988). However, the loss of cells with the whey is less than the increase in cell numbers due to growth during production. Therefore there is a net increase in cells in the curd above the concentration effect of curd formation (Buazzi *et al*., 1992). The loss of pathogenic cells to whey during manufacture is modelled using the data of Papageorgiou and Marth (1989a) for *L. monocytogenes*, which is assumed to be an appropriate estimate for all three pathogens.

Raw milk has a pH of between 6.5 - 6.7. During cheese production, the starter culture produces organic acids from the sugars present in the milk. Figure 2 shows pooled data for observed changes in pH in Feta cheese from the start of manufacture (Day 0) until the end of ripening (Day 90). In the first day there is a rapid decline in pH followed by stabilisation of pH at around 4.6 (Papageorgiou and Marth, 1989a). Slight changes in the production steps do not produce large changes in the pH during development of the cheese. However the rate of change in the pH is dependent on the temperature at which the cheese is stored (Govaris, 2002). At a higher storage temperature the pH falls faster than at a lower storage temperature.

3.2 Ripening

The ripening stage of Feta cheese production influences the survival of pathogenic bacteria that may be present in the cheese. For the three organisms modelled a gradual inactivation has been observed during this stage of manufacture (Papageorgiou and Marth, 1989a; Govaris et al, 2002; Erkmen, 1995). To model this process, the concentration of each pathogen in the hooped curds is taken and for each day of ripening an inactivation model is used to estimate the decline in population of the pathogens. The temperature of ripening is not constant between different manufacturing processes for Feta cheese. Govaris et al (2002) use 16ºC as the initial temperature for ripening. Once the cheese has reached the appropriate pH (in this case 4.6) the temperature is lowered to 4ºC. However, Papageorgiou and Marth (1989a) use 4ºC as the ripening temperature irrespective of the pH of the cheese at the end of manufacture. There is insufficient data to make a temperature and pH dependent model for ripening and subsequently it is assumed in the model that ripening occurs at 4ºC for 90 days.

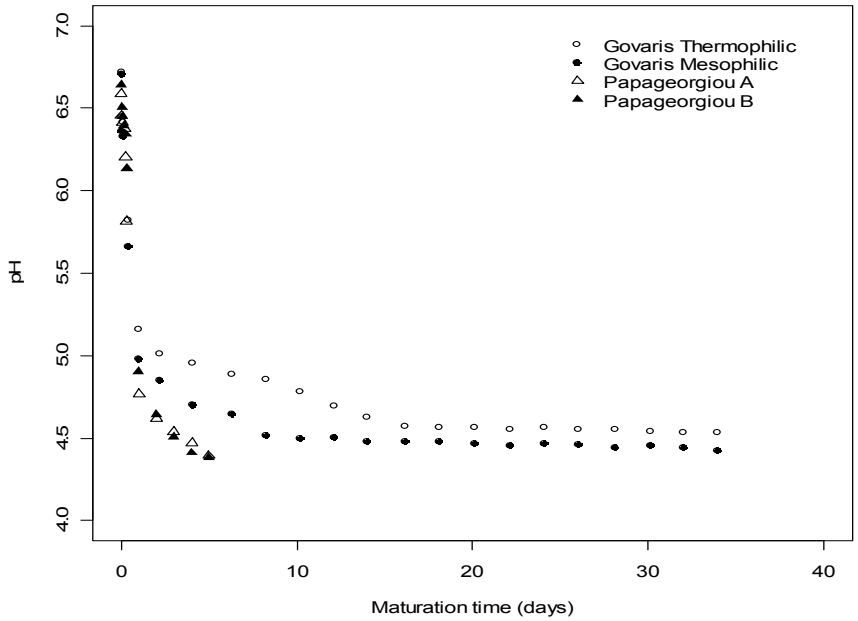

Figure 2: Changes that occur in the pH of Feta cheese during ripening. Data adapted from Govaris et al. (2002) and Papageorgiou and Marth (1989a)

3.2.1 Effect of ripening on *E. coli*

E. coli has been shown to decrease in numbers during the ripening stage of Feta production. Govaris *et al*. (2002) showed complete inactivation of *E. coli* (to below detectable limits) within 40 days (Figure 3). Data of Manolopoulou *et al*. (2003) showed an initial increase in the number of *E. coli* in the cheese in the first 10 days followed by a decrease in numbers, with no cells detected at 120 days. The increase in concentration of *E. coli* in Feta cheese observed by Manolopoulou *et al*. (2003) during the initial stage of manufacture was between 2.2 - 3.8 log, whereas Govaris *et al.* (2002) observed an increase of only between 0.82 - 1.2 log.

The rate of inactivation is temperature dependent and varies depending on the type of starter culture used. At higher temperatures the rate of inactivation of *E. coli* is faster than at a lower temperature (Govaris, 2002). Using 4ºC as the temperature for ripening the decimal reduction times for *E. coli* was modelled using a triangular distribution with minimum, mean and maximum values determined through linear regression of the inactivation curves in the 4ºC portion. The decimal reduction times were found to be between 14.85 - 16.03 days.

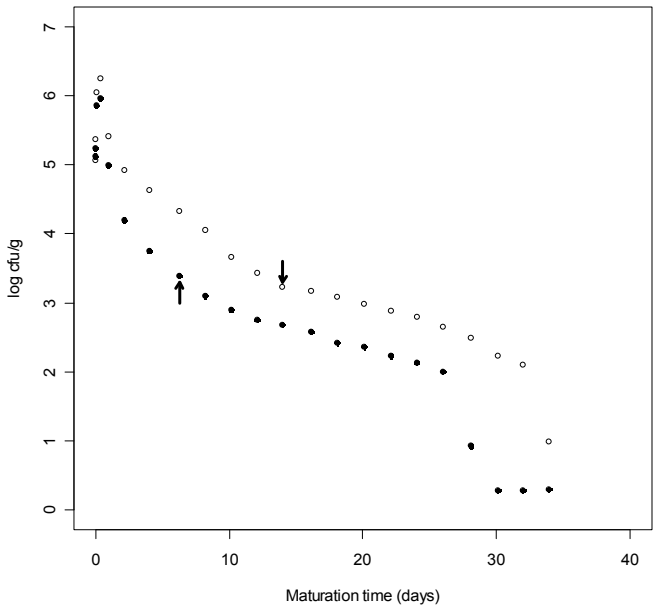

Figure 3: Effect of maturation on concentration of *E. coli* (Govaris, 2002). Arrows indicate the change in temperature from 16ºC to 4ºC for cheeses manufactured with mesophilic (filled circles) and thermophilic (open circles) starter cultures

3.2.2 Effect of ripening on *L. monocytogenes*

During ripening of Feta cheese, *L. monocytogenes* also exhibits a decline in numbers, however the rate of inactivation is much slower than for *E. coli*. Papageorgiou and Marth (1989a) examined the fate of two strains of *L. monocytogenes* during Feta production and ripening, Scott A (a clinical isolate) and CA (an isolate from Mexican style cheese).

In Feta cheese ripened at 4ºC in a 6% brine solution, *L. monocytogenes* Scott A has been shown to survive better than the CA strain. However, both strains had viable counts after 90 days (Papageorgiou and Marth, 1989a)(Figure 4). Linear regression was used to determine the rate of inactivation during maturation for each of the trials. In order to capture

the differences in survival behaviour between strains, statistical distributions were developed to define the decimal reduction time for both Scott A and CA (California) strains. Each distribution was considered equally likely for the development of the predictive model. The observed decrease in *L. monocytogenes* during ripening of Feta cheese is between 0.77 - 2.77 log cfu/g.

Papageorgiou and Marth (1989a) also present data for the survival of *L. monocytogenes* in the brine solution used during ripening. For the Scott A strain, the pattern of survival was similar, however for the CA strain, there was greater survival in the brine than in the cheese (Figure 5). The decimal reduction times in brine were in the order of 44.6 and 92 days for CA and Scott A strains, respectively.

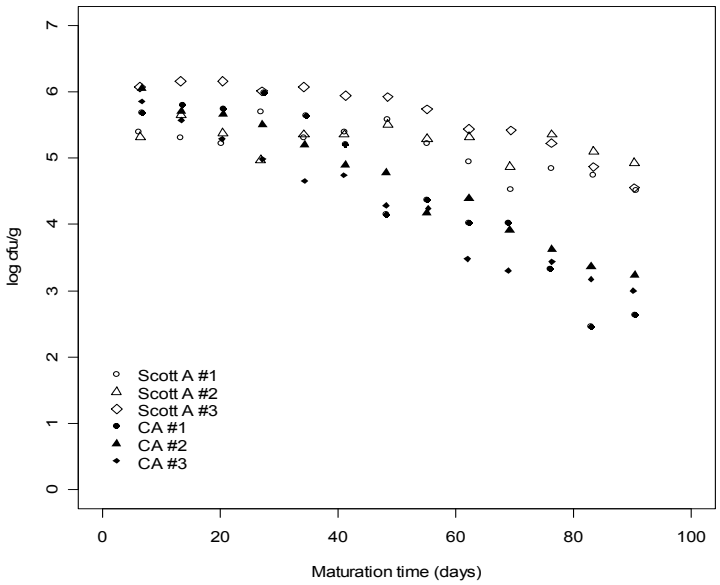

Figure 4: Effect of ripening (4ºC) on *L. monocytogenes* Scott A and CA in Feta cheese (Papageorgiou and Marth, 1989a)

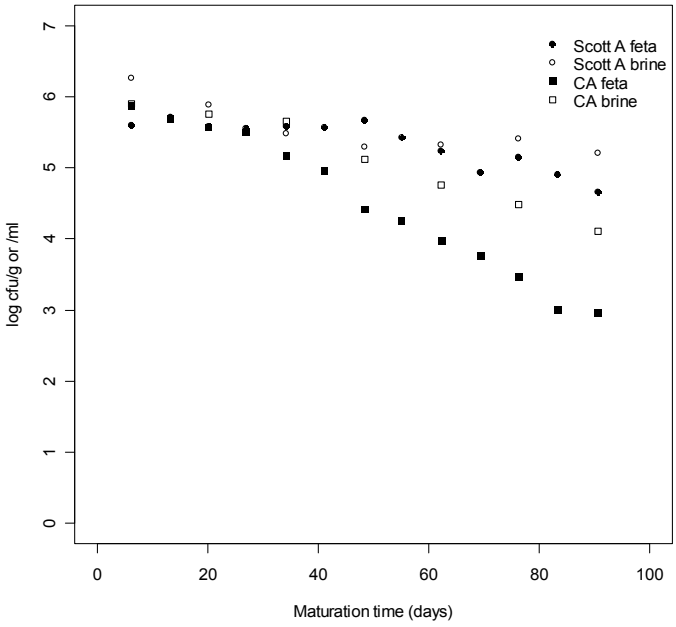

Figure 5: Survival of *L. monocytogenes* in 6% brine solution used during ripening (4ºC) of Feta cheese (Papageorgiou and Marth, 1989a)

3.2.3 Effect of ripening on *S. aureus*

During the initial fermentation phase of Feta cheese there is growth of *S. aureus* followed by a decrease in numbers during maturation. The rate of inactivation does not appear to be dependent on the concentration of salt used during brining the cheese (Erkmen, 1995). Data presented by Erkmen (1995) show that the decimal reduction times for *S. aureus* ranged between 18.0 and 24.8 days at 4ºC, using linear regression from the highest concentration of cells in the cheese (Figure 6). Average decimal reduction time was found to be 21.6 days at 4ºC.

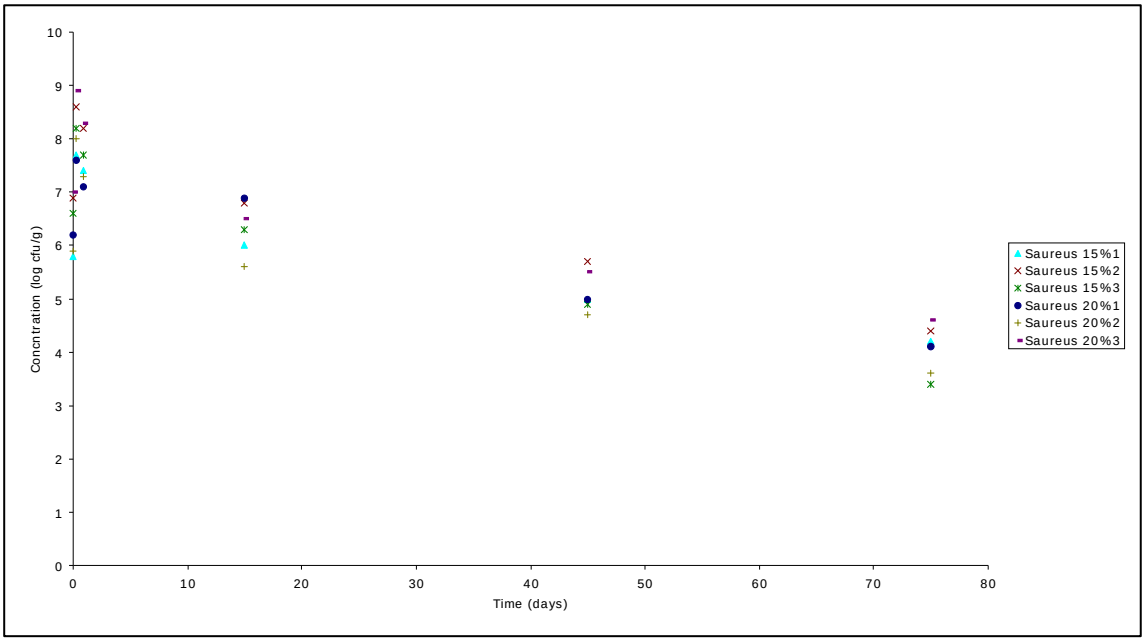

Figure 6: Changes in the population of *S. aureus* during raw milk Feta cheese production and ripening at 4ºC (Erkmen, 1995)

As previously discussed, the important aspect of *S. aureus* presence in cheese is not the final concentration, rather the maximum population achieved during production, as this has the greatest impact on the levels of enterotoxins that may be produced (Meyrand, 1998). The level of concern for this organism is considered to be between 10^5 and 10^6 cfu/g (Lindqvist *et al*., 2002).

3.3 Model set up

The model was repeatedly run with different initial pathogen contamination in milk to determine an initial starting concentration for each pathogen that would meet the microbiological limits in the *Australia New Zealand Food Standards Code* (the Code). Each simulation was run using Latin hypercube sampling of distributions until convergence was achieved. The criteria for convergence was a less than 1.5% change in the mean values of the outputs, updated every 5000 iterations.

During the hooping of soft curds into frames prior to the draining of whey the model assumes that there is a large pool of milk used for production, rather than a finite volume. This reduces the complexity of the model and a Poisson process may be assumed rather than modelling using a hypergeometric function. In this way, individual cheeses are modelled

from this point onwards, whereas the initial stages of production are in reference to the total volume of milk being processed.

3.4 Probabilistic model results

During the production of Feta cheese there is the possibility that pathogens present in the milk may be able to survive and grow. The period of potential growth is from the addition of the starter culture to just prior to the beginning of ripening (approximately 24 hours).

3.4.1 *E. coli*

The estimated growth of *E. coli* during the initial production phase (prior to ripening) for raw milk Feta cheese was 5.3 log (Standard deviation, s.d. ±0.47). Govaris *et al.* (2002) reported that during the first 10 hours of cheese manufacture *E. coli* O157:H7 grew between 0.82 and 1.18 log. For a similar time period, between the start of production and draining the whey, the model estimated that *E. coli* would grow between 2.2 - 3.1 log with an average increase of 2.7 log. This indicates that the model without a lag phase estimated faster growth rates, or more growth, for *E. coli* than has been observed in laboratory studies.

For *E. coli*, the average inactivation during ripening was estimated to be 3.3 log. This compares with the observed inactivation of between 5 - 5.5 log during Feta ripening (Govaris, 2002). These authors found that *E. coli* was not detectable after 56 days of ripening. Differences in the modelled estimate and the observed inactivation are due to the length of ripening and the method used to model the inactivation.

The final concentration of *E. coli* in 90 day matured raw milk Feta cheese decreased on average by approximately two orders of magnitude compared with the initial contamination in milk. For example, starting with an initial contamination of 1 cell/ml in milk, the average estimated final concentration was 1.37 cells/g of cheese. An equivalent decrease was predicted for the other initial concentrations (Table 2).

Table 2: Simulation estimates for the concentration of *E. coli* in raw milk Feta cheese after 90 days ripening from different initial contamination concentrations

	Initial contamination in milk				
	0.001 cell/ml	0.1 cell/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	8.82×10^{0}	8.82×10^{2}	8.94×10^{3}	7.92×10^{4}	8.73×10^{5}
Mean	4.67×10^{2}	4.67×10^{4}	4.61×10^{5}	4.66×10^{6}	4.66×10^{7}
Maximum	1.54×10^{5}	1.54×10^{7}	6.43×10^{7}	1.47×10^{9}	3.22×10^{10}
5th percentile	4.75×10^{1}	4.75×10^{3}	4.76×10^{4}	4.77×10^{5}	4.79×10^{6}
95th percentile	1.56×10^{3}	1.56×10^{5}	1.56×10^{6}	1.56×10^{7}	1.55×10^{8}
End of 90 day maturation concentration in cheese (cells/g)					
Minimum	6.62×10^{-6}	6.62×10^{-4}	4.85×10^{-3}	6.84×10^{-2}	4.37×10^{-1}
Mean	1.37×10^{-3}	1.37×10^{-1}	1.37×10^{0}	1.38×10^{1}	1.37×10^{2}
Maximum	9.17×10^{-1}	9.17×10^{1}	3.84×10^{2}	7.14×10^{3}	4.20×10^{4}
5th percentile	7.15×10^{-5}	7.15×10^{-3}	7.14×10^{-2}	7.04×10^{-1}	7.07×10^{0}
95th percentile	4.75×10^{-3}	4.75×10^{-1}	4.82×10^{0}	4.82×10^{1}	4.77×10^{2}

Table 2 cont: Simulation estimates for the concentration of *E. coli* in raw milk Feta cheese after 90 days ripening from different initial contamination concentrations

	Initial contamination in milk				
	0.001 cell/ml	0.1 cells/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	8.82×10^{0}	8.82×10^{2}	8.94×10^{3}	7.92×10^{4}	8.73×10^{5}
Mean	4.67×10^{2}	4.67×10^{4}	4.61×10^{5}	4.66×10^{6}	4.66×10^{7}
Maximum	1.54×10^{5}	1.54×10^{7}	6.43×10^{7}	1.47×10^{9}	3.22×10^{10}
5th percentile	4.75×10^{1}	4.75×10^{3}	4.76×10^{4}	4.77×10^{5}	4.79×10^{6}
95th percentile	1.56×10^{3}	1.56×10^{5}	1.56×10^{6}	1.56×10^{7}	1.55×10^{8}
End of 90 day maturation concentration in cheese (cells/g)					
Minimum	6.62×10^{-6}	6.62×10^{-4}	4.85×10^{-3}	6.84×10^{-2}	4.37×10^{-1}
Mean	1.37×10^{-3}	1.37×10^{-1}	1.37×10^{0}	1.38×10^{1}	1.37×10^{2}
Maximum	9.17×10^{-1}	9.17×10^{1}	3.84×10^{2}	7.14×10^{3}	4.20×10^{4}
5th percentile	7.15×10^{-5}	7.15×10^{-3}	7.14×10^{-2}	7.04×10^{-1}	7.07×10^{0}
95th percentile	4.75×10^{-3}	4.75×10^{-1}	4.82×10^{0}	4.82×10^{1}	4.77×10^{2}

3.4.2 *S. aureus*

During the initial production of raw milk Feta, the model estimated that the average growth of *S. aureus* was 4.2 log (s.d. ±0.23). Observed increases in the concentration of *S. aureus* during raw milk Feta manufacture were between 1.8 and 2.3 log (Erkmen, 1995). The model substantially overestimated the growth of *S. aureus* during the manufacture of raw milk Feta cheese. This difference may be due in part to no inclusion of a lag phase in the model.

For *S. aureus*, the average estimated inactivation during ripening was 5.6 log. Erkmen (1995) observed that *S. aureus* was inactivated by 3.6 log on average within the range of 3 and 4.3 log. The difference between the estimated inactivation and observed inactivation is due to the time of ripening, with Erkmen (1995) following survival over 75 days, and the model using 90 days ripening. By using 75 days as the ripening time in the model, the average estimated inactivation for *S. aureus* was 4.6 log.

The estimated final concentration of *S. aureus* in raw milk Feta cheese ripened for 90 days changed on average 0.1 log compared with the initial contamination in milk. Starting with an initial contamination of 1 cell/ml in raw milk, the average estimated concentration in 90 day ripened raw milk Feta was 7.45×10^{-2} cell/g of cheese, *i.e.* an overall reduction in the concentration of *S. aureus* by around 1 log (Table 3).

Table 3: Simulation estimates for the concentration of *S. aureus* in raw milk Feta cheese after 90 days ripening from different initial contamination concentrations

	Initial contamination in milk				
	0.01 cell/ml	0.1 cell/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	8.82×10^{0}	8.82×10^{2}	9.29×10^{3}	9.17×10^{4}	9.22×10^{5}
Mean	2.76×10^{1}	2.76×10^{3}	2.76×10^{4}	2.76×10^{5}	2.76×10^{6}
Maximum	8.61×10^{1}	8.61×10^{3}	7.92×10^{4}	8.20×10^{5}	7.81×10^{6}
5th percentile	1.59×10^{1}	1.59×10^{3}	1.58×10^{4}	1.58×10^{5}	1.59×10^{6}
95th percentile	4.39×10^{1}	4.39×10^{3}	4.39×10^{4}	4.40×10^{5}	4.40×10^{6}
End of 90 day maturation concentration in cheese (cells/g)					
Minimum	2.68×10^{-13}	2.68×10^{-11}	7.46×10^{-10}	3.75×10^{-9}	2.21×10^{-8}
Mean	7.55×10^{-5}	7.55×10^{-3}	7.45×10^{-2}	7.50×10^{-1}	7.53×10^{0}
Maximum	1.77×10^{-2}	1.77×10^{0}	1.36×10^{1}	8.59×10^{1}	1.31×10^{3}
5th percentile	5.25×10^{-9}	5.25×10^{-7}	5.16×10^{-6}	4.86×10^{-5}	5.13×10^{-4}
95th percentile	3.78×10^{-4}	3.78×10^{-2}	3.68×10^{-1}	3.66×10^{0}	3.70×10^{1}

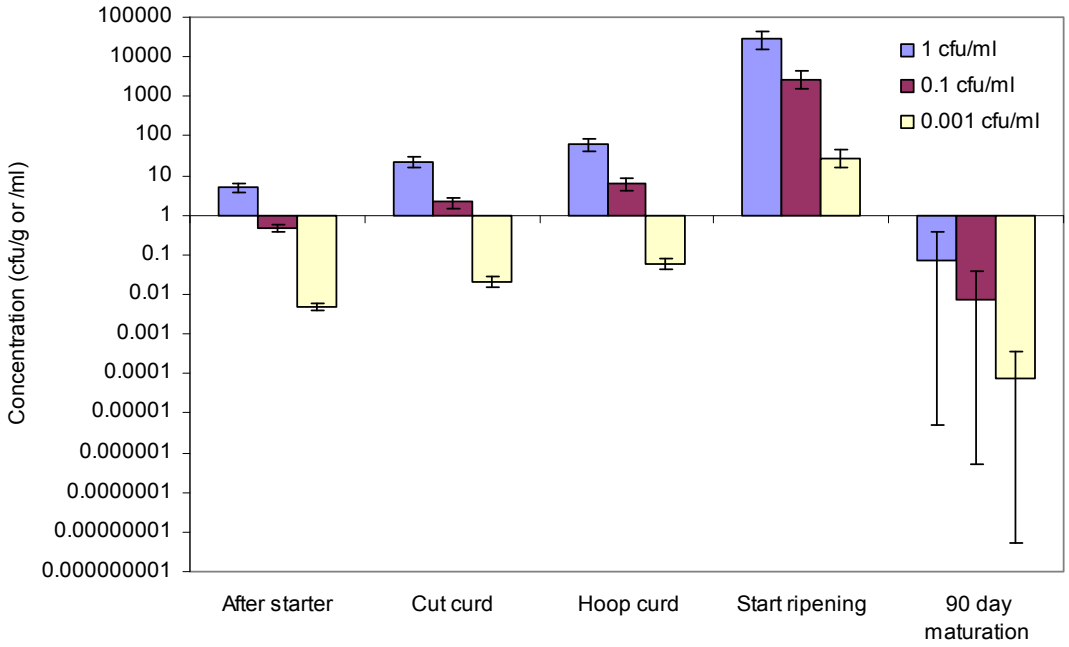

Figure 7: Average estimated concentration of *S. aureus* during production of raw milk Feta cheese with different initial starting concentration in milk. Error bars indicate the 5th and 95th percentile values

Figure 7 shows the average concentration of *S. aureus* at different stages of raw milk Feta production. The highest population level reached is 4.4 log cfu/g in the cheese just prior to ripening from milk with an initial contamination level of 1 cell/ml.

3.4.3 *L. monocytogenes*

The estimated growth of *L. monocytogenes* during initial stages of raw milk Feta production was 2.4 log (s.d. ± 0.13). This compares well with the growth of 2.35 log (s.d. ± 0.12) observed by Papageorgiou and Marth (1989a). This accounts for the concentration effect of curd formation, growth during manufacture and the loss of cells to whey during drainage.

The situation for *L. monocytogenes* is complex due to large differences in the tolerance of different strains to ripening conditions of raw milk Feta cheese. *L. monocytogenes* Scott A is a clinical isolate of the organism and has relatively high tolerance to the ripening conditions of raw milk Feta cheese compared with the CA strain, an isolate from Mexican style cheese, where the inactivation rate is faster. The average estimated inactivation during 90 days of ripening of raw milk Feta cheese is estimated at 2.7 log, with 5^{th} and 95^{th} percentile values of 5.4 and 0.3 log, respectively. Observed average inactivation for Scott A and CA during Feta production are 0.8 and 2.8, respectively (Papageorgiou and Marth, 1989a).

For *L. monocytogenes* the net change between the start of production and the end of 90 day ripening was estimated to be approximately 0.7 log. Starting with an initial concentration of 1 cell/ml in milk, the average estimated concentration in 90 day ripened raw milk Feta cheese is 242 cells/g, an overall increase in the concentration of 2.38 logs. A similar increase can be seen with other initial starting concentrations (Table 4). The apparent difference in the net change (0.7 logs) and the concentration (2.38 logs) is due to the asymmetry of the

concentration distribution. The mean value is located at the 75th percentile, compared to the 50th percentile of a symmetrical distribution.

Table 4: Simulation estimates for the concentration of *L. monocytogenes* in raw milk Feta cheese after 90 days ripening from different initial contamination concentrations

	Initial contamination in milk				
	0.001 cell/ml	0.1 cell/ml	1 cell/ml	10 cells/ml	100 cells/ml
Start of maturation concentration in cheese (cells/g)					
Minimum	1.08×10^{0}	1.08×10^{2}	1.10×10^{3}	1.11×10^{4}	1.06×10^{5}
Mean	2.59×10^{0}	2.59×10^{2}	2.59×10^{3}	2.59×10^{4}	2.59×10^{5}
Maximum	7.23×10^{0}	7.23×10^{2}	8.18×10^{3}	8.11×10^{4}	7.45×10^{5}
5th percentile	1.55×10^{0}	1.55×10^{2}	1.56×10^{3}	1.56×10^{4}	1.56×10^{5}
95th percentile	4.25×10^{0}	4.25×10^{2}	4.24×10^{3}	4.24×10^{4}	4.25×10^{5}
End of 90 day maturation concentration in cheese (cells/g)					
Minimum	0	0	0	0	0
Mean	2.45×10^{-1}	2.45×10^{1}	2.42×10^{2}	2.43×10^{3}	2.43×10^{4}
Maximum	4.78×10^{0}	4.78×10^{2}	4.55×10^{3}	5.59×10^{4}	4.79×10^{5}
5th percentile	8.85×10^{-6}	8.85×10^{-4}	9.18×10^{-3}	8.80×10^{-2}	9.50×10^{-1}
95th percentile	1.33×10^{0}	1.33×10^{2}	1.31×10^{3}	1.32×10^{4}	1.32×10^{5}

3.5 Sensitivity analysis

Regression sensitivity analysis was performed on the model for each of the pathogens at the 90 day ripening stage to determine which variables had the greatest influence on the final concentration in raw milk Feta cheese. Regardless of the initial contamination level in the milk used to produce raw milk Feta, the model estimates have the same sensitivity to each variable in the model. For some model inputs a distribution of values was not available and were therefore modelled using fixed values (for example the initial decrease in pH during manufacture). The sensitivity analyses only consider those inputs described using distributions. The standardised b coefficient (= slope) provides an indication of the strength of the linear relationship between the input variable and the output variable. As general 'rule-of-thumb', values of the standardised b coefficient > 0.5 are considered to be important.

Figures 8, 9 and 10 show the regression sensitivity for each of the pathogens *E. coli*, *L. monocytogenes* and *S. aureus* respectively. No factors were identified as being significant on the final concentration of *E. coli* in the raw milk Feta cheese. For both *L. monocytogenes* and *S. aureus* the most important factor was the decimal reduction time. The standardised b coefficients were found to be 0.728 and 0.684 for *L. monocytogenes* and *S. aureus,* respectively. In addition, for *L. monocytogenes* the binomial distribution selecting between the decimal reduction time distributions for the Scott A and CA strains was also found to be important.

The results of the sensitivity analysis should be treated with caution. This approach indicates the relative strength between an input and output variable based on the assumption that the relationship is linear. There may be non-linear relationships between variables that would not be identified using this approach.

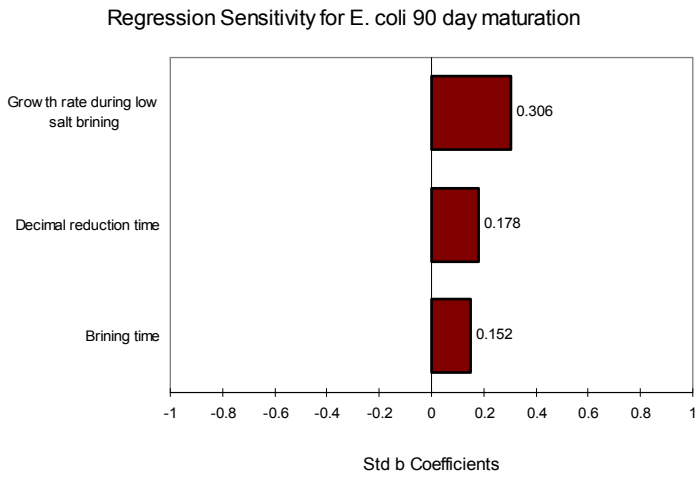

Figure 8: Regression sensitivity for the final concentration of *E. coli* in 90 day ripened raw milk Feta cheese

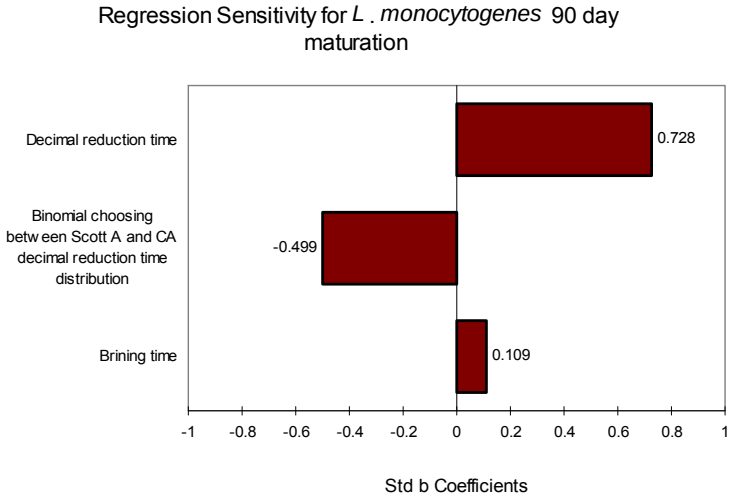

Figure 9: Regression sensitivity for the final concentration of *L. monocytogenes* in 90 day ripened raw milk Feta cheese

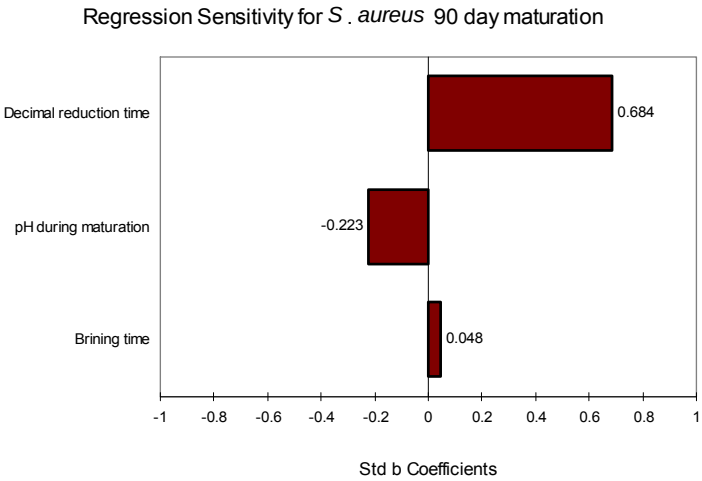

Figure 10: Regression sensitivity for the final concentration of *S. aureus* in 90 day ripened raw milk Feta cheese

3.6 Summary of modelled results on effect of manufacture

The mean log change in numbers of organisms during the initial manufacture and subsequent ripening are provided in Table 5.

For *E. coli*, there was an estimated net decrease of 1.5 log during the production of raw milk Feta cheese. The regulatory requirement for *E. coli* is for no sample to contain in excess of 100 cfu/g of cheese. According to the model, raw milk Feta cheese produced from milk contaminated with 100 cells/ml will not exceed 100 cfu/g of cheese. Moreover the probability of exceeding 10 cfu/g in raw milk Feta is less than 10%.

The primary concern with *S. aureus* is the likelihood of the organism growing to high enough numbers to produce sufficient toxin to cause illness in humans. The model estimated that during production of raw milk Feta using milk with an initial starting concentration of 10 cfu/ml, levels would reach 10^5 cfu/g prior to ripening. A 4 log reduction during ripening resulted in a final concentration of approximately 10 cfu/g in the cheese.

For *L. monocytogenes* the regulatory limit is for no sample to contain detectable levels of the organism in 25grams of cheese. Using the conservative model estimates for *L. monocytogenes* Scott A, a concentration of less than 10^{-3}/ml in raw milk would mean that detection of the organism is unlikely (<4% of iterations greater than the limit).

A summary of the estimated impact on the concentration of specific microorganisms during the production of raw milk Feta cheese is summarised in Table 5.

Table 5: Predicted changes in concentration of microorganisms during raw milk Feta cheese production (using Log 10 scale)

	E. coli	***S. aureus***	***L. monocytogenes***
First 24 hours	+5.60	+4.44	+3.41
Maturation	-5.52	-5.57	-1.03
Net Change	**0.14**	**-1.13**	**+2.38**

3.7 Consumption of Feta cheeses in Australia

Data from the 1995 Australian National Nutrition Survey[48] (NNS) gives an indication of the percentage of the population who consume various types of cheese and the amount they consume.

Feta cheese is not commonly consumed in Australia with data from the NNS indicating that only 0.6% of those surveyed consumed Feta cheese with the average amount consumed being 41g (Table 6). Table 6 shows data from the NNS on the Australian average daily consumption of Feta cheese by gender and age. Consumption of Feta cheese varies across age groups, however children under the age of 3 were not reported to consume this cheese.

[48] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24 hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

It cannot be assumed that this same proportion of the population would also consume raw milk cheese. However it is likely that those who will consume raw milk cheese, will not increase their cheese consumption, rather they will substitute consumption of pasteurised cheese with raw milk cheese. Therefore this data indicates consumption of raw milk Feta cheese, if available, is likely to be extremely low.

Table 6: Australian average daily consumption Feta cheese by gender and age (Australian Government Department of Health and Family Services, 1997).

Gender	Age	No. of respondents	No. of consumers (% of respondents)		Average amount of cheese consumed (g/day)
Male	2 - 3	170	0		0
Male	4 - 7	416	3	(0.7)	25
Male	8 - 11	385	1	(0.3)	8
Male	12 - 15	349	0		0
Male	16 - 18	215	0		0
Male	19 - 24	485	4	(0.8)	31
Male	25 - 44	2140	16	(0.7)	46
Male	45 - 64	1554	10	(0.6)	54
Male	65+	902	3	(0.3)	54
Female	2 - 3	213	0		0
Female	4 - 7	383	3	(0.8)	13
Female	8 - 11	354	0		0
Female	12 - 15	304	0		0
Female	16 - 18	218	1	(0.5)	78
Female	19 - 24	575	5	(0.9)	76
Female	25 - 44	2385	18	(0.8)	45
Female	45 - 64	1752	19	(1.1)	32
Female	65+	1058	3	(0.3)	37

4 Risk characterisation

In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles (Appendix 1).

The qualitative framework considers the characterisation of identified hazards (hazard identification and characterisation combined) and an assessment of the likely exposure to these hazards (exposure assessment) which when combined provides a characterisation of the risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of disease. The exposure module characterises exposure to the hazard based on the likely level of the hazard in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of the hazard on a "per serve" basis[49]. Essentially the matrix categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

The model was employed to characterise the risk from raw milk Feta cheese.

[49] "per serve" is defined as the amount of product consumed per eating occasion.

Risk categories for hazards in raw milk Feta cheese were assigned as follows:

Hazard	Infective dose	Consequence of exposure	Severity of hazard
E. coli (EHEC)	<10	Serious	High
S. aureus	>1,000	Mild	Negligible
L. monocytogenes	>1,000/10-100#	Moderate/Severe#	Negligible/Moderate#

susceptible populations

Exposure categories for raw milk Feta cheese:

Pathogen	Raw product contamination	Effect of processing	Exposure
E. coli (EHEC)	Infrequent (1%)	No effect	Low*
S. aureus	Sometimes (10%)	No effect	Moderate
L. monocytogenes	Infrequent (1%)	10 fold increase	Moderate

* For raw milk cheese made from sheep milk the raw milk contamination is Sometimes (10%) resulting in an exposure of Moderate

Risk characterisation for raw milk Feta cheese:

Pathogen	Hazard charcterisation	Exposure assessment	Risk Characterisation
E. coli (EHEC)	High	Low	High
S. aureus	Negligible	Moderate	Low
L. monocytogenes	Negligible/Moderate#	Moderate	Low/High#

susceptible populations

5 Conclusions

During the production of Feta it was estimated that the overall concentration of *E. coli* remained largely unchanged from that observed in the raw milk, while there was an increase of 2.38 log in the concentration of *L. monocytogenes* from the initial levels in the raw milk to those present in the final cheese. For *S. aureus* there was a slight decrease in the overall concentration of approximately 1.13 log.

Challenge studies by Ramsaran *et al.* (1998) showed *E. coli* to survive the manufacturing process of Feta cheese. Levels in the final cheese were 1.33 log greater than that in the initial inoculum (Ramsaran, 1998). Govaris (2002) however showed complete inactivation (5 and 5.5 log) of *E. coli* (to below detectable limits) within 40 days. Data of Manolopoulou *et al.* (2003) showed an initial increase in the number of *E. coli* in the cheese during the first 10 days followed by a decrease in numbers, with no cells detected at 120 days (Figures.3 and 4). The increase in concentration of *E. coli* during the initial manufacture in Feta cheese observed by Manolopoulou *et al*. (2003) was between 2.2 - 3.8 log, whereas Govaris (2002) only observed an increase of between 0.82 - 1.2 log.

The rate of inactivation of *E. coli* in Feta cheese also appears to be temperature dependent. At higher temperatures the rate of inactivation is faster than at a lower temperature (Govaris, 2002).

Papageorgiou and Marth (1989a) examined the fate of two strains of *L. monocytogenes* during Feta production and ripening and found both strains had viable counts after 90 days (Papageorgiou and Marth, 1989a). Ramsaran *et al.* (1998) also report an overall increase

(~1 log) in numbers of *L. monocytogenes* during manufacture and ripening of Feta cheese (Ramsaran, 1998).

The process of manufacturing raw milk Feta cheese has been assessed to affect selected pathogens as follows:

Pathogen	Risk associated with raw milk Feta Cheese
E. coli (EHEC)	**High** risk as *E. coli* survives during cheesemaking
S. aureus	Risk from staphylococcal enterotoxin is considered **low**. Conditional on good control over animal health and raw milk handling.
L. monocytogenes	**Low** risk (general population) and **high** risk (susceptible population groups) as the organism survives and increases in levels in cheesemaking process.

It is considered there is little difference in the public health and safety risk from *L. monocytogenes* and *S. aureus* in raw milk Feta cheeses made from either cow, goat or sheep milk.

The microbiological safety of raw milk Feta cheese appears to be largely dependent upon the microbiological quality of the raw milk and rapid acidification (*i.e.* <5.0 within 6 - 8 hours and ~ 4.8 after 18 - 20 hours).

Quantitative modelling has shown that in order to produce raw milk Feta cheese that would meet current microbiological limits in the *Australia New Zealand Food Standards Code*, the initial concentration of *E. coli*, and *L. monocytogenes* in the raw milk would need to less than 1 and 10^{-5} cfu/ml respectively. In order to produce Feta cheese unlikely to contain sufficient staphylococcal toxin to cause illness (*i.e.* $<10^5$ cfu/g), the initial concentration of cells in the raw milk would need to be less than 10^3 cfu/ml.

The findings of the modelled raw milk Feta cheese assessed cannot be applied to other semi-soft cheeses based on moisture (*i.e.* 43 - 55%) as the modelled cheeses does not represent all types of semi-soft cheeses in respect to physicochemical characteristics and manufacturing protocols. The findings, however, may be applied to other Feta cheeses whose manufacturing specifications lie within the specification range of the modelled cheese.

Appendix 13: Risk assessment – Raw milk Camembert cheese

1 Introduction

Camembert cheese is characterised by surface ripening by the moulds *Penicillium camemberti and Penicillium candidum*. Coagulation is achieved using rennet and is acidified using mesophilic starter cultures. The coagulated curd is ladled directly into moulds for draining. Camembert cheeses are brine salted and initially ripened for 10 - 12 days at 12°C to enable mould formation, followed by storage at ~4°C for ~30 days.

Camembert cheeses are generally classified as "soft" cheese, the typical characteristics of which are outlined in Appendix 3.

The Codex standard for Camembert cheese[50] contains details on the principal characteristics of this cheese (such as appearance, texture and origin of milk), and specifies a maximum moisture content of 56 - 62 % and a maturation/curing period of 10 days at 10 - 14°C possibly followed by storage at lower temperatures.

This risk assessment examines the fate of Enterohaemorragic *Escherichia coli*, *Staphylococcusaureus* and *Listeria monocytogenes* during the manufacture of a raw milk Camembert cheese based on a probabilistic model developed by the University of Tasmania and adapted by FSANZ.

The manufacturing parameters and physicochemical properties for the modelled raw milk Camembert cheese are based on experimental data and do not necessarily reflect commercial manufacturing practices. The modelled raw milk Camembert cheese manufacturing parameters and physicochemical characteristics are described in Figure 1.

A qualitative framework was subsequently used to rate the risk to public health and safety from the consumption of raw milk Camembert cheese made from cow, goat or sheep milk, containing these microbiological hazards.

2 Hazard identification and hazard characterisation

In evaluating the safety of raw milk Camembert cheese, the following pathogens were considered: *E. coli*, *L. monocytogenes*, and *S. aureus*. A detailed characterisation of potential hazards is attached as Appendix 14.

3 Exposure assessment

To simulate the fate of pathogens during the production of raw milk Camembert cheese a model was developed from published descriptions of Camembert cheese production (Back *et al*., 1993; Helloin, 2003; Leclercq-Perlat *et al*., 2004; Meyrand, 1998; Ramsaran, 1998). A conceptual flow diagram of the production process is given (Figure 1) and the steps and variables involved in raw milk Camembert manufacture are listed in Table 1. There is a degree of diversity in the production times and temperatures used for the production of Camembert cheese. These differences are encompassed in the time and temperature distributions used in the model. Predominantly a uniform distribution is used to model the

[50] Codex International Standard for Camembert Cheese, *CODEX STAN 33-1973*

variations in the value of a particular parameter, due to a lack of data concerning the relative weight that a particular value may have over another.

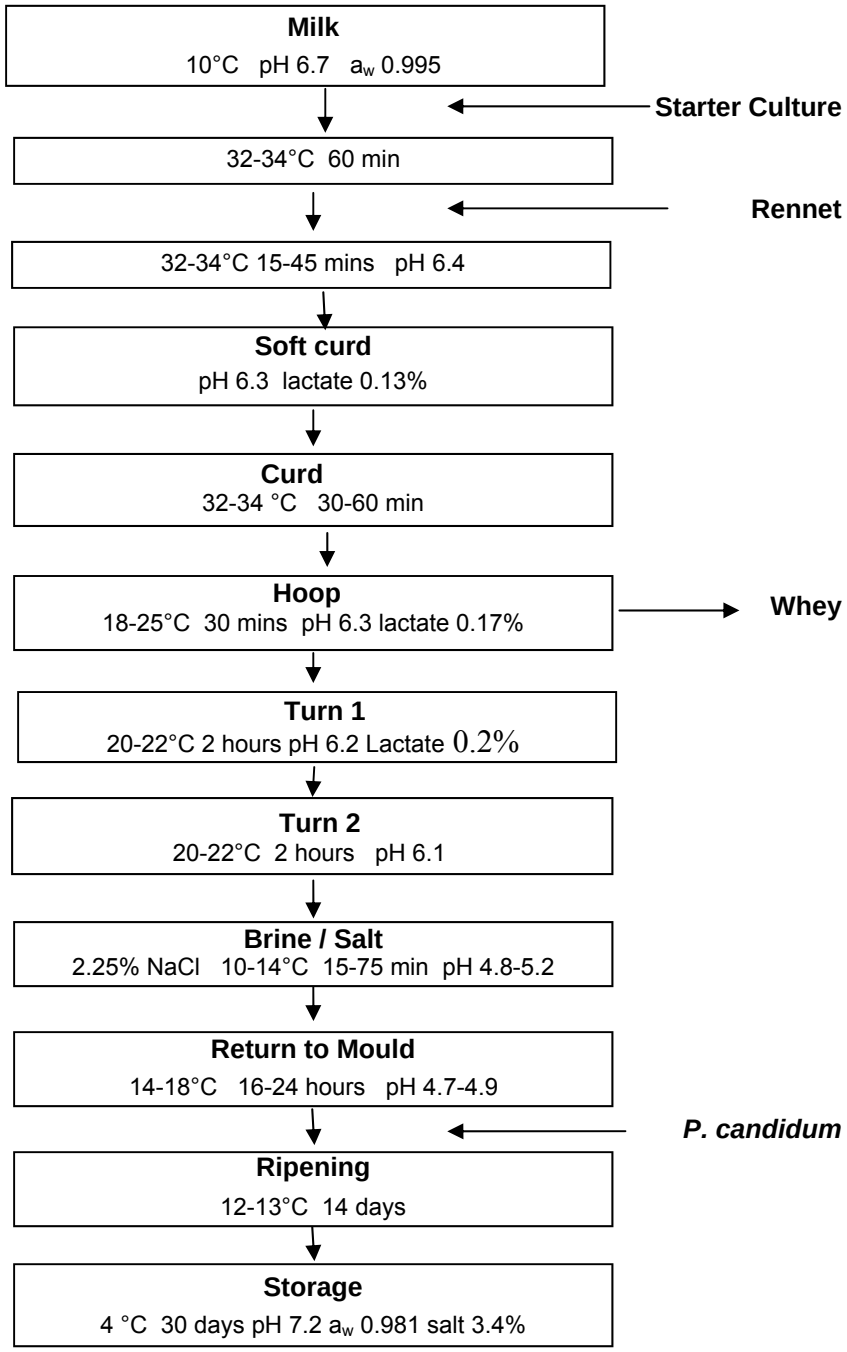

Figure 1: Conceptual flow diagram for the production of raw milk Camembert cheese

Table 1: Processing steps involved in the production of raw milk Camembert cheese and the distribution functions for variables in each step

Step	Parameter	Distribution function
Add starter	Temperature (ºC) Time (min)	Uniform (32, 34) 60
Add rennet	Temperature (ºC) Time (min)	Uniform (32, 34) Uniform (15, 45)
Cut curd	Temperature (ºC) Time (min)	Uniform (32, 34) Uniform (30, 60)
Hoop curd	Temperature (ºC) Time (min)	Uniform (18, 25) 30
Whey Drained	% cells lost	Normal (3.21, 2.18) Truncated at 0
1st turn	Temperature (ºC) Time (hours)	Uniform (20, 22) 2
2nd turn	Temperature (ºC) Time (hours)	Uniform (20, 22) 2
Brine/Salt	Temperature (ºC) pH water activity/salt Time (min)	Uniform (10, 14) Uniform (4.8, 5.2) 0.986 / 2.25% Uniform (15, 75)
Return to mould	Temperature Time (hours) pH	Uniform (14, 18) Uniform (16, 24) Uniform (4.7, 4.9)
Ripening	Time (days) Temperature (ºC)	14 Uniform (12, 14)
Storage	Time (days) Temperature (ºC)	30 days 4

3.1 Initial manufacture phase

Growth of starter culture and non-starter bacteria during initial fermentation phase has an impact on the growth potential of pathogens that may be present in the milk. The effects of competition between pathogenic bacteria and starter culture bacteria have not been modelled, however the impact of changes in the pH caused by lactic acid bacteria is modelled.

The loss of pathogenic cells to whey during manufacture was modelled using the data of Papageorgiou and Marth (1989a) for *L. monocytogenes*, which is assumed to be an appropriate estimate for all three pathogens and for this type of cheese.

The growth rates of pathogenic bacteria in the cheese are estimated using the growth models of Buchanan *et al.* (1993), Murphy *et al.* (1996) and Ross *et al.* (2003) as detailed in Appendix 2. A second growth model for *L. monocytogenes* was implemented for this cheese that accounts for the lactic acid concentration (Ross and Soontranon, 2006). The model has previously been cited in a publication comparing different growth rate models for *L. monocytogenes* in cold smoked salmon (Dalgaard, 1998). The effect of lactic acid on the potential growth of *L. monocytogenes* has a large impact on the estimates for the concentration in the finished product. There are no known growth rate models available that describe the effect of lactic acid on *S. aureus*.

For a conservative estimate of potential contamination in the finished product it is assumed there is no lag phase during production.

3.2 Ripening

The ripening stage of Camembert cheese production significantly influences the survival of pathogenic bacteria that may be present in the cheese.

The physicochemical composition of Camembert cheese changes during production from growth inhibiting to growth permissive at different stages of ripening because of the growth of the surface moulds. This is due to changes in pH, lactic acid concentration and temperature. Initially the curds have even and consistent properties with respect to pH and lactic acid. However, as the cheese ripens changes in the pH, salt concentration and lactic acid concentration become evident (Addis, 2001; Leclercq-Perlat *et al*., 2004; Meyrand, 1998) see Figure 2.

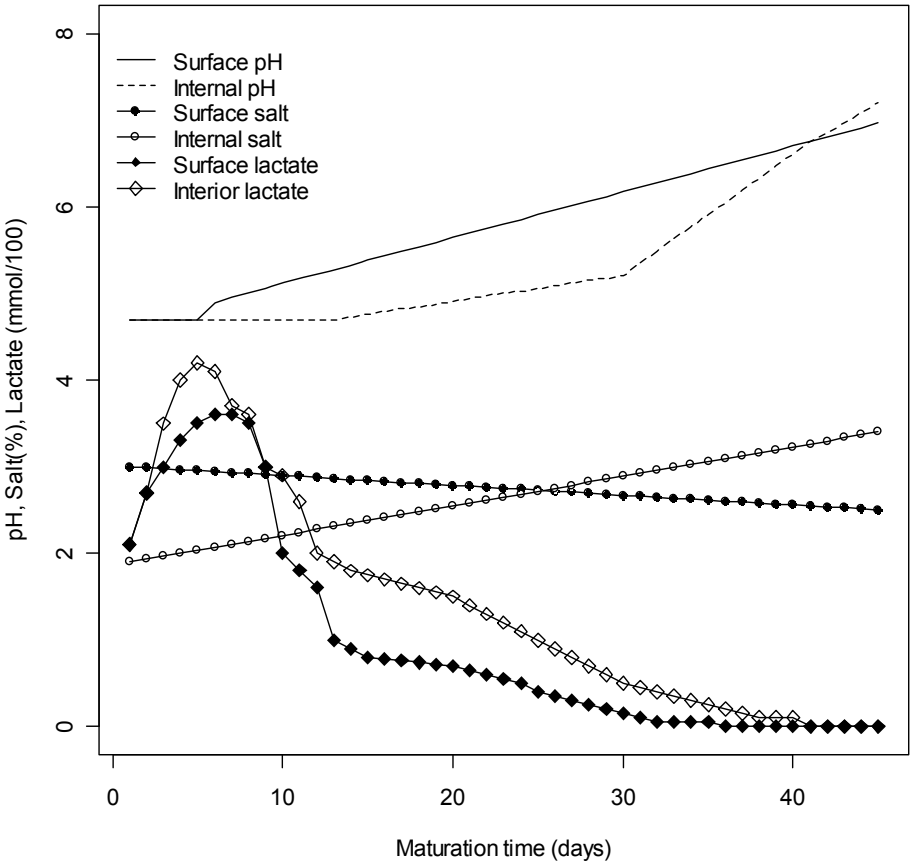

Figure 2 : Changes in the pH, salt and lactic acid concentration between the surface and centre of Camembert cheese during ripening (Adapted from (Addis, 2001; Back *et al*., 1993; Guizani *et al*., 2002; Leclercq-Perlat *et al*., 2004; Meyrand, 1998))

During the first 14 days of ripening the temperature is maintained between 12 - 13ºC. At this temperature growth of all organisms is possible. However, the lactic acid concentration inhibits the growth of *E. coli* until day 11 when it falls to a level where growth is possible at the surface, but not the centre of the cheese where the fall in concentration of lactic acid is slower. After 14 days the temperature is reduced to 4ºC, which prevents the growth of both *E. coli* and *S. aureus*.

For *L. monocytogenes* the Murphy model predicts that growth may occur throughout the entire ripening and storage phases for Camembert cheese (Murphy, 1996). There was a difference in the observed and predicted growth rates in Camembert cheese stored at 4ºC, where the predicted growth rate was approximately double that observed. This has been accounted for in the risk assessment model by halving the growth rate predictions in the maturation phase. When the Ross and Soontranon model for *L. monocytogenes* is used, growth is inhibited in the first 12 days on the surface of the cheese and 28 days internally due to the effect of lactic acid present in the cheese (Ross and Soontranon, 2006). Incorporating these predictions into the model changed the final predicted concentration in the cheese by several orders of magnitude.

For *E. coli* and *S. aureus*, although storage conditions after 14 days are not conducive to growth, it is assumed that during this time there is limited inactivation or reduction in numbers.

3.3 Model setup

The model was developed to predict the concentration of pathogenic bacteria at the surface and the centre of the cheese from raw milk through to the end of the maturation phase. The predictions of the surface and centre pathogen concentrations were then combined to determine the concentration of an equivalent wedge of cheese, as typically consumed. The wedge provides a weighted estimate of the relative contributions of the surface and interior growth of the pathogens. It is assumed that the interior core of the cheese is 10% of the total mass. Furthermore, a comparison between the model estimates using the Murphy and the Ross and Soontranon models for *L. monocytogenes* is presented.

3.4 Probabilistic model results

During ripening, the conditions allow the potential growth of the three pathogens. *E. coli* and *S. aureus* grow in the first 14 days when the temperature is above 10ºC, and *L. monocytogenes* has the potential to grow during the entire period. The net result is an increase in the concentration of all pathogens in the cheese by the end of the storage period (14 days ripening at 12ºC and 31 days storage at 4ºC). However, the presence of lactate in the cheese acts as an inhibitor to growth. Consequently, only the growth models that incorporate a lactic acid term will provide meaningful results as to the potential growth during ripening and storage.

The model estimates indicate that there is a lower concentration of the pathogens in the centre of the cheese than at the surface.

3.4.1 *E. coli*

The average estimated growth of *E. coli* during the manufacture of raw milk Camembert cheese is 2.3 log. This is independent of the initial contamination in the milk used for manufacture. Ramsaran *et al.* (1998) observed an increase in the concentration of *E. coli* during the first 24 hours of Camembert production of approximately 2 log.

For *E. coli*, the estimated mean growth during ripening and storage was 1.5 log for the surface of the cheese and 0 log for the internal part of the cheese (Table 2). The growth internally was so low because the level of lactate in the cheese does not decrease to a level

where *E. coli* may grow prior to the temperature being changed from 12ºC to 4ºC (Leclercq-Perlat *et al*., 2004). Observed changes in the concentration of *E. coli* during ripening and storage show a decline in numbers of approximately 1 log during 65 days at 2ºC, however, no distinction was made between counts at the surface and centre of the cheese (Ramsaran, 1998).

There was a net increase in the concentration of *E. coli* in raw milk Camembert cheese for the surface of 3 log and a net increase of 2 log for the internal portion of the cheese.

Table 2: Simulation estimates for the concentration of *E. coli* at the start of ripening and for internal, surface and wedge concentrations after 45 days ripening and storage from different initial contamination concentrations

	Initial contamination in milk			
	0.001 cell/ml	0.01 cell/ml	0.1 cell/ml	1 cell/ml
Start of maturation concentration in cheese (cells/g)				
Minimum	7.19×10^{-2}	6.88×10^{-1}	7.24	7.01×10^{1}
Mean	2.37×10^{-1}	2.37	2.37×10^{1}	2.37×10^{2}
Maximum	8.77×10^{-1}	8.71	9.73×10^{1}	9.74×10^{2}
5th percentile	1.22×10^{-1}	1.22	1.22×0^{1}	1.22×10^{2}
95th percentile	4.16×10^{-1}	4.19	4.7×10^{1}	4.19×10^{2}
End of 45 day maturation concentration on the surface of raw milk Camembert (cells/g)				
Minimum	1.53	1.52×10^{1}	1.86×10^{2}	1.57×10^{3}
Mean	7.34	7.36×10^{1}	7.35×10^{2}	7.35×10^{3}
Maximum	3.50×10^{1}	3.67×10^{2}	3.51×10^{3}	4.09×10^{4}
5th percentile	3.49	3.49×10^{1}	3.50×10^{2}	3.49×10^{3}
95th percentile	1.35×10^{1}	1.36×10^{2}	1.35×10^{3}	1.36×10^{4}
End of 45 day maturation concentration on the interior of raw milk Camembert (cells/g)				
Minimum	7.19×10^{-2}	7.08×10^{-1}	7.02	7.01×10^{1}
Mean	2.37×10^{-1}	2.37	2.37×10^{1}	2.37×10^{2}
Maximum	8.77×10^{-1}	9.86	9.35×10^{1}	9.74×10^{2}
5th percentile	1.22×10^{-1}	1.22	1.23×10^{1}	1.22×10^{2}
95th percentile	4.16×10^{-1}	4.19	4.16×10^{1}	4.19×10^{2}
End of 45 day maturation concentration for a wedge of raw milk Camembert (cells/g)				
Minimum	1.38	1.38×10^{1}	1.68×10^{2}	1.42×10^{3}
Mean	6.6	6.65×10^{1}	6.64×10^{2}	6.64×10^{3}
Maximum	3.16×10^{1}	3.31×10^{2}	3.17×10^{3}	3.69×10^{4}
5th percentile	3.15	3.15×10^{1}	3.16×10^{2}	3.15×10^{3}
95th percentile	1.22×10^{1}	1.23×10^{2}	1.22×10^{3}	1.23×10^{4}

3.4.2 *S. aureus*

The estimated average increase in *S. aureus* prior to ripening of raw milk Camembert cheese was 2.9 log. This compares well with the observed increase of approximately 3 log during the first 22 hours of production for Camembert cheese with initial inoculum levels between 10^2 - 10^6 (Meyrand, 1998).

The average estimated growth of *S. aureus* during ripening and storage was 4.5 log at the surface and 3.1 log at the centre of the cheese. Observed changes in *S. aureus* counts during ripening and storage of Camembert cheese show a gradual reduction in numbers over 41 days of approximately 1 log (Meyrand, 1998). It was assumed that during storage of raw milk Camembert at 4ºC there was no decline in *S. aureus* numbers, however, there was insufficient information to model the decline suggested by Meyrand . (1998). Addis *et al.* (2001) observed a slight decline in *Staphylococcus* spp. numbers in the inner curd of Camembert together with approximately 2 log increase at the surface during 75 day ripening and storage. The temperature of ripening and storage phase was not specified in the paper.

Final estimated concentrations of *S. aureus* on the cheese surface and the inner curd were 7 logs and 6 logs greater, respectively, than those initially present in the raw milk used for cheesemaking. Furthermore the maximum concentration that the organism reached during cheesemaking is of importance due to the possibility of toxin production. Figure 4 shows the estimated concentration of *S. aureus* at different stages of cheese production.

Table 3: Simulation estimates for the concentration of *S. aureus* at the start of ripening and for internal, surface and wedge concentrations after 45 day ripening and storage from different initial contamination concentrations

	Initial contamination in milk			
	0.001 cell/ml	0.01 cell/ml	0.1 cell/ml	1 cell/ml
Start of maturation concentration in cheese (cells/g)				
Minimum	1.91×10^{-1}	1.82	1.88×10^{1}	1.85×10^{2}
Mean	8.50×10^{-1}	8.51	8.49×10^{1}	8.50×10^{2}
Maximum	3.95	4.56×10^{1}	3.91×10^{2}	3.81×10^{3}
5th percentile	3.70×10^{-1}	3.70	3.75×10^{1}	3.72×10^{2}
95th percentile	1.64	1.64×10^{1}	1.63×10^{2}	1.64×10^{3}
End of 45 day maturation concentration on the surface of raw milk Camembert (cells/g)				
Minimum	4.26×10^{3}	4.82×10^{4}	5.19×10^{5}	4.38×10^{6}
Mean	3.02×10^{4}	3.03×10^{5}	3.02×10^{6}	3.03×10^{7}
Maximum	1.74×10^{5}	1.85×10^{6}	1.79×10^{7}	1.59×10^{8}
5th percentile	1.21×10^{4}	1.20×10^{5}	1.22×10^{6}	1.20×10^{7}
95th percentile	6.09×10^{4}	6.11×10^{5}	6.08×10^{6}	6.14×10^{7}
End of 45 day maturation concentration on the interior of raw milk Camembert (cells/g)				
Minimum	1.93×10^{2}	2.09×10^{3}	2.18×10^{4}	1.99×10^{5}
Mean	1.09×10^{3}	1.09×10^{4}	1.09×10^{5}	1.09×10^{6}
Maximum	5.85×10^{3}	5.85×10^{4}	5.24×10^{5}	4.89×10^{6}
5th percentile	4.55×10^{2}	4.55×10^{3}	4.61×10^{4}	4.57×10^{5}
95th percentile	2.13×10^{3}	2.14×10^{4}	2.11×10^{5}	2.14×10^{6}
End of 45 day maturation concentration for a wedge of raw milk Camembert (cells/g)				
Minimum	3.85×10^{3}	4.36×10^{4}	4.69×10^{5}	3.96×10^{6}
Mean	2.73×10^{4}	2.74×10^{5}	2.73×10^{6}	2.74×10^{7}
Maximum	1.57×10^{5}	1.67×10^{6}	1.62×10^{7}	1.44×10^{8}
5th percentile	1.09×10^{4}	1.08×10^{5}	1.10×10^{6}	1.08×10^{7}
95th percentile	5.50×10^{4}	5.52×10^{5}	5.49×10^{6}	5.55×10^{7}

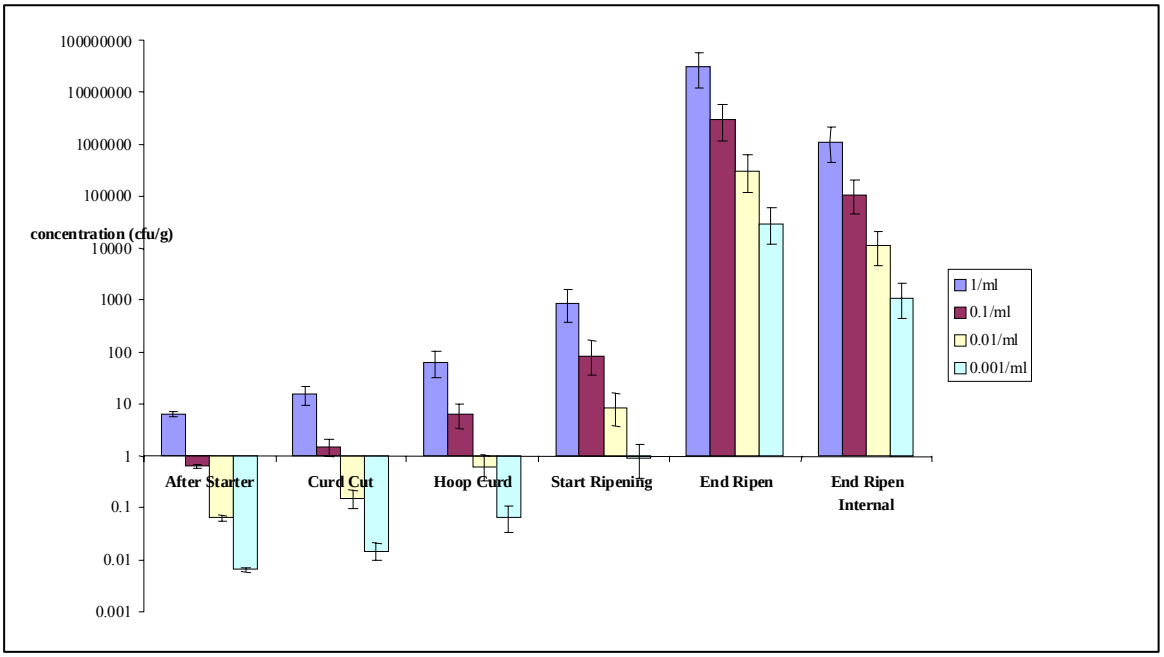

Figure 4: Estimated concentration of *S. aureus* at different stages of raw milk Camembert cheese manufacture with different initial contamination levels in milk

3.4.3 *L. monocytogenes*

For *L. monocytogenes* the estimated average growth during manufacture was between 2.4 and 2.7 log using the Murphy and the Ross and Soontranon models respectively. Both models predict greater growth than has been observed, with reported growth of approximately 1 log (Ramsaran, 1998).

There is a significant difference in the estimated growth of *L. monocytogenes* between the two models used. The Murphy model does not contain a term for lactic acid, and produces extreme estimates for potential growth, whereas the Ross and Soontranon model does contain a lactic acid term and its estimates are much lower during ripening and storage.

The Murphy model estimated growth during ripening and storage of 14.1 log and 7.9 log for the surface and inner curd, respectively. The use of a maximum population density (MPD) in the model would have resulted in the majority of samples reaching the MPD and estimated growth would have been relative to the MPD level. However, when using the Ross and Soontranon model which incorporates a term for lactate, the estimated growth during ripening and storage was much lower, 3.7 log and 1.7 log for surface and inner curd respectively. Ramsaran *et al.* (1998) observed an increase in the concentration of *L. monocytogenes* of between 1 - 1.5 log. Whereas, Back *et al.* (1993) observed increases of between 1 - 4 log on the surface of the cheese over 42 days, and an increase of 2 log or a decline of less than 1 log in the inner curd, depending on the temperature of storage. These results are similar to those observed by Ryser and Marth (1987a). After a decline of up to 2 logs in the concentrations at the surface, interior and the wedge sample the concentration increased as the pH increased above 6. The amount of growth in the interior of the cheese was delayed and also slower than observed on the surface of the cheese.

For more realistic estimates for the concentration of *L. monocytogenes* in raw milk Camembert cheese at the end of storage and maturation, the Ross and Soontranon model was used for the final estimates.

There was a net increase of *L. monocytogenes* from the initial concentration in the milk to that in the finished cheese. Overall the average increase between the initial contamination in milk and the finished product was 6 log and 4 log for the surface and inner curd, respectively.

Table 4: Simulation estimates for the concentration of *L. monocytogenes* at the start of ripening and for internal and surface concentrations after 45 day ripening and storage from different initial contamination concentrations

	Initial contamination in milk			
	0.001 cell/ml	0.01 cell/ml	0.1 cells/ml	1 cell/ml
Start of maturation concentration in cheese (cells/g) †				
Minimum	1.62×10^{-1}	1.50	1.64×10^{1}	1.63×10^{2}
Mean	4.86×10^{-1}	4.86	4.86×10^{1}	4.86×10^{2}
Maximum	1.26	1.28×10^{1}	1.34×10^{2}	1.32×10^{3}
5th percentile	2.64×10^{-1}	2.62	2.62×10^{1}	2.63×10^{2}
95th percentile	8.03×10^{-1}	8.03	8.07×10^{1}	8.06×10^{2}
End of 45 day maturation concentration on the surface of raw milk Camembert (cells/g) †				
Minimum	5.29×10^{2}	5.74×10^{3}	5.88×10^{3}	5.59×10^{5}
Mean	2.37×10^{3}	2.36×10^{4}	$2.36x\ 10^{5}$	2.37×10^{6}
Maximum	8.71×10^{3}	9.35×10^{4}	9.13×10^{5}	1.18×10^{7}
5th percentile	1.16×10^{3}	1.16×10^{4}	1.15×10^{5}	1.15×10^{6}
95th percentile	4.20×10^{3}	4.16×10^{4}	4.18×10^{5}	4.19×10^{6}
End of 45 day maturation concentration on the interior of raw milk Camembert (cells/g) †				
Minimum	8.26	7.99×10^{1}	8.07×10^{2}	8.38×10^{3}
Mean	2.48×10^{1}	2.49×10^{2}	2.48×10^{3}	2.48×10^{4}
Maximum	6.45×10^{1}	6.96×10^{2}	6.96×10^{3}	7.11×10^{4}
5th percentile	1.35×10^{1}	1.34×10^{2}	1.35×10^{3}	1.35×10^{4}
95th percentile	4.11×10^{1}	4.10×10^{2}	4.09×10^{3}	4.12×10^{4}
End of 45 day maturation concentration for a wedge of raw milk Camembert (cells/g) †				
Minimum	4.77×10^{2}	5.17×10^{3}	5.37×10^{3}	5.04×10^{5}
Mean	2.14×10^{3}	2.13×10^{4}	2.13×10^{5}	2.14×10^{6}
Maximum	7.85×10^{3}	8.42×10^{4}	8.22×10^{5}	1.06×10^{7}
5th percentile	1.05×10^{3}	1.05×10^{4}	1.04×10^{5}	1.04×10^{6}
95th percentile	3.78×10^{3}	3.75×10^{4}	3.77×10^{5}	3.78×10^{6}

† Estimates are based on the growth model of Ross and Soontranon.

3.5 Sensitivity analyses

Regression sensitivity analysis was performed on the model for each of the pathogens at the end of ripening to determine which variables had the greatest influence on the final concentration in raw milk Camembert cheese. For some model inputs a distribution of values was not available and were therefore modelled using fixed values (for example the initial decrease in pH during manufacture). The sensitivity analyses only consider those inputs described using distributions. Regardless of the initial contamination level in the milk, the model estimates have the same sensitivity to different variables.

For each pathogen there are two sensitivity graphs, one for the surface estimates and one for the inner curd. The regression sensitivity for *E. coli* for the surface and centre of the cheese are shown in Figures 5 and 6, respectively. Figures 7 and 8 show the regression sensitivity for *S. aureus* for the surface and centre, and Figures 9 and 10 are for *L. monocytogenes*. In each case the majority of the variables in the model have a positive impact on the final concentration of the organisms. There are only, at most, two variables that negatively impact on the final concentration, these being the loss of cells during whey drainage and the surface pH of the cheese during ripening.

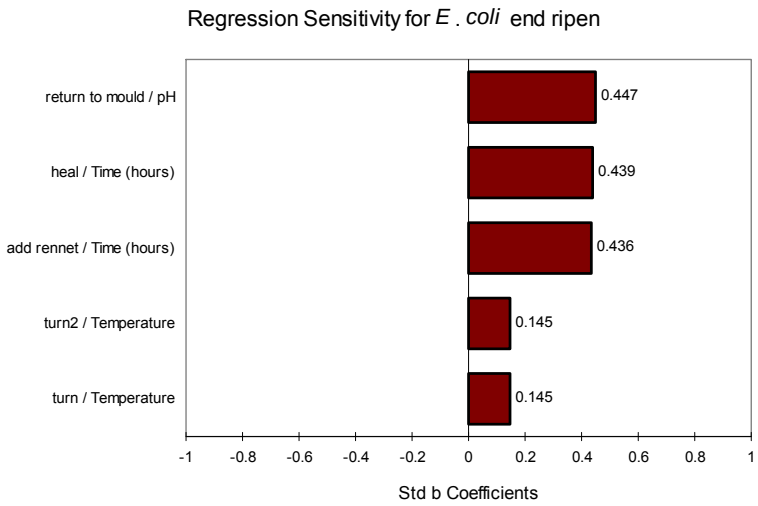

Figure 5: Regression sensitivity results for *E. coli* at the surface of raw milk Camembert cheese at the end of ripening

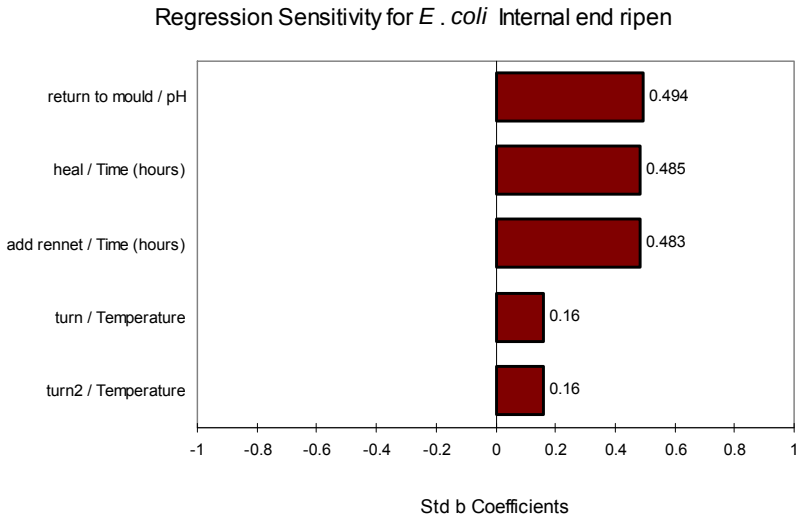

Figure 6: Regression sensitivity results for *E. coli* at the centre of raw milk Camembert cheese at the end of ripening

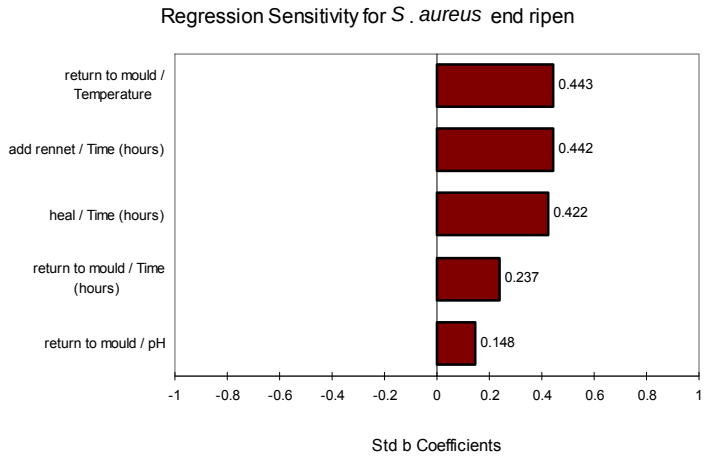

Figure 7: Regression sensitivity results for *S. aureus* at the surface of raw milk Camembert cheese at the end of ripening

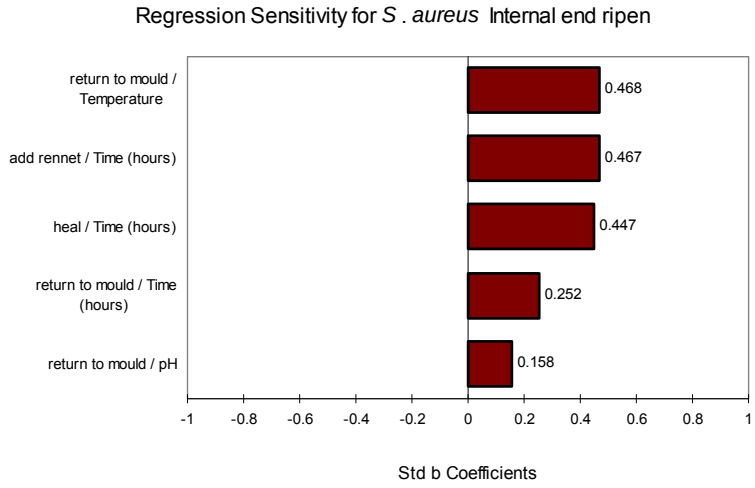

Figure 8: Regression sensitivity results for *S. aureus* at the centre of raw milk Camembert cheese at the end of ripening

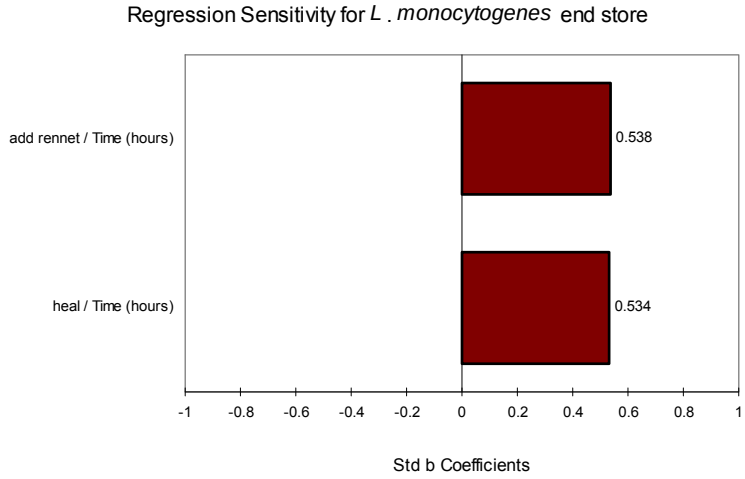

Figure 9: Regression sensitivity results for *L. monocytogenes* at the surface of raw milk Camembert cheese at the end of ripening

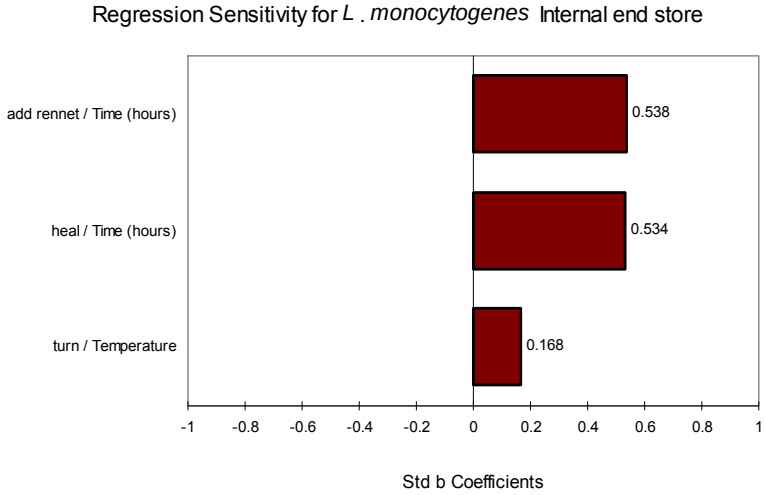

Figure 10: Regression sensitivity results for *L. monocytogenes* at the centre of raw milk Camembert cheese at the end of ripening

3.6 Summary of modelled results on effect of manufacture

Results from the quantitative models were used to predict the probability that cheese made from milk with different levels of contamination was to meet current requirements in the *Australia New Zealand Food Standards Code* (the Code). For each organism, the effect of production and maturation of raw milk Camembert cheese is different and subsequently the maximum concentration at which each organism may be present in milk is also different (Figures 11 and 12).

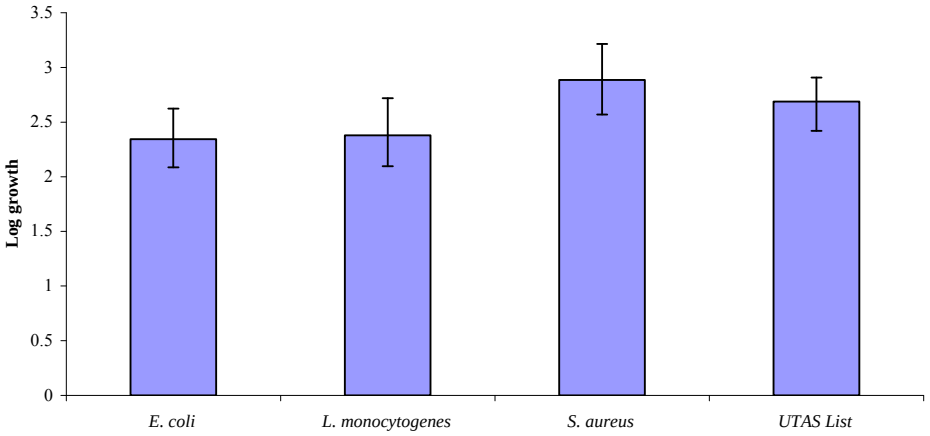

Figure 11: Summary of estimated growth of *E. coli*, *L. monocytogenes* and *S. aureus* prior to ripening of raw milk Camembert cheese. Error bars indicate the 5th and 95th percentile values

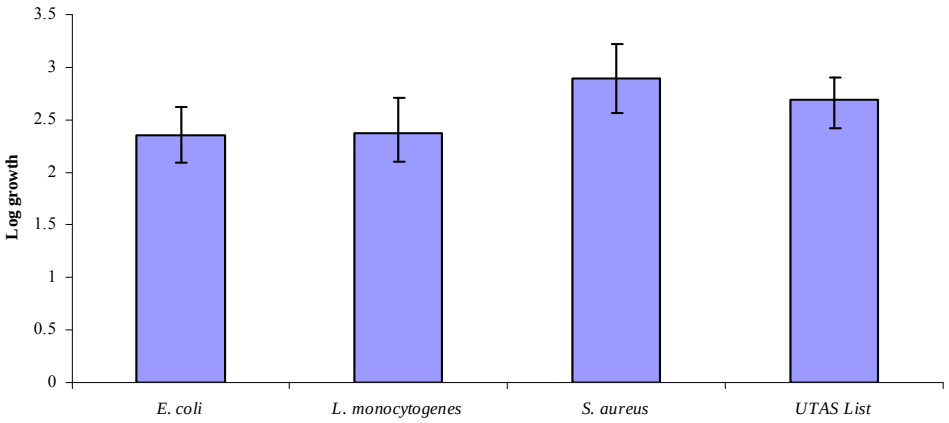

Figure 12: Summary of average estimated growth during ripening and storage of raw milk Camembert cheese for *E. coli*, *S. aureus* and *L. monocytogenes*. There was no predicted growth of *E. coli* for the inner curd. Error bars indicate the 5th and 95th percentile values.

For *E. coli* the regulatory requirement is that no sample contains greater than 100 cfu/g of cheese. According to the model estimates, milk contaminated with 0.001 cells/ml of *E. coli*, will produce a cheese where the surface contamination does not exceed the regulatory limit. At this level there were approximately 85% of iterations where the final concentration was greater than 10 cells/g in the cheese. For the centre of the cheese, milk containing 0.01 cell/ml and was not estimated to exceed the lower limit of 10 cells/g. However, with an

initial contamination of 0.1 cell/ml greater than 95% of iterations produced estimates higher than 10 cells/g of cheese.

For *S. aureus* the model estimated that an initial contamination of less than 0.1 cell/ml in the raw milk would not allow the population to reach the threshold for toxin production.

The requirement for *L. monocytogenes* is for no detection of the organism in five samples of 25g. The model estimates indicated that to make raw milk Camembert cheese that meets these requirements the concentration in raw milk would need to be much less than 10^{-5} cell/ml (less than 1 cell in 100 L of milk).

A summary of the estimated impact on the concentration of specific microorganisms during the production of raw milk Camembert cheese is summarised in Table 5.

Table 5: Predicted changes in concentration of microorganisms during raw milk Camembert cheese production

Raw milk Camembert	Log_{10} change conc.	*E. coli*	*S. aureus*	*L. monocytogenes*
- surface	First 24 hours	+2.37	+2.93	+2.69
	Maturation	+1.49	+4.55	+3.69
	Net Change	**+3.87**	**+7.48**	**+6.37**
- internal				
	Maturation	0	+3.11	+1.71
	Net Change	**+2.37**	**+6.04**	**+4.39**
- wedge				
	Maturation	+1.44	+4.51	+3.64
	Net Change	**+3.81**	**+7.44**	**+6.33**

3.7 Consumption of Camembert cheeses in Australia

Data from the 1995 Australian National Nutrition Survey[51] (NNS) gives an indication of the percentage of the population who consume various types of cheese and the amount they consume.

Camembert cheese is not commonly consumed in Australia with data from the NNS indicating that less than 1% of those surveyed consumed Camembert/Brie cheeses, with the average amount consumed being 35 g (Table 6). Table 6 shows data from the NNS on the Australian average daily consumption of Camembert/Brie cheese by gender and age. There was no reported consumption for people under the age of 15 and the highest consumption amount was by males aged 19-24 who consumed an average 68 g.

It cannot be assumed that this same proportion of the population would also consume raw milk cheese. However, it is likely that those who will consume raw milk cheese, will not increase their cheese consumption, rather they will substitute consumption of pasteurised cheese with raw milk cheese.

[51] Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey. Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24 hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

Table 6: Australian average daily consumption of Camembert (including Brie) cheese by gender and age (Australian Government Department of Health and Family Services, 1997)

Gender	Age	No. of respondents	No. of consumers (% of respondents)		Average amount of cheese consumed (g/day)
Male	2 - 3	170	0		0
Male	4 - 7	416	0		0
Male	8 - 11	385	0		0
Male	12 - 15	349	0		0
Male	16 - 18	215	0		0
Male	19 - 24	485	1	(0.1)	68
Male	25 - 44	2140	15	(0.7)	51
Male	45 - 64	1554	14	(0.9)	23
Male	65+	902	3	(0.3)	17
Female	2 - 3	213	0		0
Female	4 - 7	383	0		0
Female	8 - 11	354	0		0
Female	12 - 15	304	0		0
Female	16 - 18	218	1	(0.5)	34
Female	19 - 24	575	3	(0.5)	31
Female	25 - 44	2385	21	(0.9)	22
Female	45 - 64	1752	23	(1.3)	33
Female	65+	1058	4	(0.4)	38

*

4 Risk characterisation

In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw milk cheeses, FSANZ has used a model developed by Food Science Australia (Vanderlinde, 2004). The approach utilises a qualitative framework based on Codex principles (Appendix 1).

The qualitative framework considers the characterisation of identified hazards (hazard identification and characterisation combined) and an assessment of the likely exposure to these hazards (exposure assessment) which when combined provides a characterisation of the risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of disease. The exposure module characterises exposure to the hazard based on the likely level of the hazard in the raw product and the effect of processing on the hazard. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of the hazard on a "per serve" basis[52]. Essentially the matrix categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

The model was employed to characterise the risk from raw milk Camembert cheese.

[52] "per serve" is defined as the amount of product consumed per eating occasion.

Risk categories for hazards in raw milk Camembert cheese were assigned as follows:

Hazard	Infective dose	Consequence of exposure	Severity of hazard
*E. coli (*EHEC*)*	<10	Serious	High
S. aureus	>1,000	Mild	Negligible
L. monocytogenes	>1,000/10-100[#]	Moderate/Severe[#]	Negligible/Moderate[#]

susceptible populations

Exposure categories for raw milk Camembert cheese:

Pathogen	Raw product contamination	Effect of processing	Exposure
E. coli (EHEC)	Infrequent (1%)	1,000 increase	High
S. aureus	Sometimes (10%)	> 1,000 increase	High
L. monocytogenes	Infrequent (1%)	>1,000 increase	High

Risk characterisation for raw milk Camembert cheese:

Pathogen	Hazard charcterisation	Exposure assessment	Risk Characterisation
E. coli (EHEC)	High	High	High
S. aureus	Negligible	High	Low
L. monocytogenes	Negligible/Moderate[#]	High	Low/High[#]

susceptible populations

5 Conclusions

For raw milk Camembert cheese, the model predicted that there are no steps during production that result in an inactivation of the microorganisms investigated, leading to a substantial increase in microorganisms during cheese production.

The ripening stage of Camembert cheese production influences the survival of pathogenic bacteria that may be present in the cheese. The physicochemical composition of Camembert cheese changes during production from growth inhibiting to growth permissive at different stages of ripening. This is due to changes in pH, lactic acid concentration and temperature. Initially the curds have even and consistent properties with respect to pH and lactic acid.

During ripening, the conditions allow the potential growth of the three pathogens. *E. coli* and *S. aureus* grow in the first 14 days, when the temperature is above 10ºC, and *L. monocytogenes* has the potential to grow during the entire period. The net result is an increase in the concentration of all pathogens in the cheese by the end of the storage period (14 days ripening at 12ºC and 31 days storage at 4ºC).

Challenge studies by Ramsaran *et al*. (1998) observed an overall increase in the concentration of *E. coli* during Camembert production of approximately 1 log (Ramsaran, 1998).

Ryser and Marth (1987a) studied the behaviour of *L. monocytogenes* in Camembert cheese. The high moisture content and the neutral pH of this surface-ripened cheese facilitate growth and survival of pathogens such as *Listeria* spp. Growth of *Listeria* spp. in Camembert cheese was found to parallel the increase in cheese pH during ripening and reached a final population of 10^6 - 10^8 per g (Ryser and Marth, 1987a). Matsusaki *et al*. (1991) also

examined the ability of *L. monocytogenes* to survive and grow in Camembert cheese during manufacture, ripening and storage. theyfound that counts typically decreased at the beginning of ripening, when the pH was lowest, then increased in the later stages of ripening and afterwards. Back *et al*. (1993) found *L. monocytogenes* survived, and under most conditions multiplied, when inoculated directly into the milk of laboratory made Camembert cheese.

Liu *et al.* (2004) examined the ability of *L. innocua* to survive and grow during ripening of Camembert cheese. Numbers increased significantly during the initial phases of cheesemaking (from 4.76 log cfu/g to 7.16 log cfu/g; then declined during the next 20 days to 6.5 log cfu/g, thereafter increasing to 7.38 log cfu/g(Liu *et al*., 2004).

Meyrand *et al.* (1998) observed changes in *S. aureus* counts during the ripening and storage of Camembert cheese with a gradual reduction in numbers over 41 days of approximately 1 log. Addis *et al*. (2001) observed a slight decline in numbers of *Staphylococcus* spp. in the inner curd of Camembert cheese together with approximately 2 log increase at the surface during 75 day ripening and storage.

The process of manufacturing raw milk Camembert cheese has been assessed to affect selected pathogens as follows:

Pathogen	Risk associated with raw milk Camembert cheese
E. coli (EHEC)	**High** risk as the organism increases in numbers during cheesemaking and maturation.
S. aureus	Risk from staphylococcal enterotoxin is considered **low**. Conditional on good control over animal health and raw milk handling. Substantial increase in levels during cheesemaking and maturation
L. monocytogenes	**Low** risk (general population) and **high** risk (susceptible population groups) as the organism increases in levels both during cheesemaking and maturation

It is considered that there is little difference in the public health and safety risk from *E. coli* (EHEC), *S. aureus* and *L. monocytogenes* in raw milk Camembert cheeses made from either cow, goat or sheep milk.

The microbiological safety of raw milk Camembert cheese appears to be largely dependent upon the microbiological quality of the raw milk and rapid acidification (*i.e.* <5.0 within 24 hours). However, subsequent changes in the physicochemical properties of the cheese, especially the increase in pH will lead to conditions conducive to the growth of pathogenic bacteria.

Quantitative modelling has shown that in order to produce raw milk Camembert that would meet current microbiological limits in the Food Standards Code, the initial concentration of *E. coli* and *L. monocytogenes* in the raw milk would need to less than 10^{-3} and 10^{-7} cfu/ml respectively. To produce Camembert cheese unlikely to have sufficient staphylococcal toxin to cause illness (*i.e.* $<10^5$ cfu/g), the initial concentration in the milk would need to be less than 10^{-4} cfu/ml.

The findings of the modelled raw milk Camembert cheese assessed may be applied to the other cheeses in the soft mould ripened category based on moisture (*i.e.* >55%) as they generally have similar physicochemical characteristics and manufacturing protocols *e.g.* minimal curd cooking, high moisture content and short ripening time.

Appendix 14: Hazard identification/hazard characterisation of pathogens

1 *Campylobacter* spp.

Campylobacter spp. are Gram-negative non-spore forming bacteria. Their cells are 0.2 - 0.8 μm wide and 0.5 - 5 μm long. They are mostly slender, spiral, curved rods, with a single polar flagellum at one or both ends of the cell. They aretypically motile with a characteristic rapid darting corkscrew-like mobility (Smibert, 1984; Vandamme, 2000) .

Campylobacter spp. are classified under *Campylobacteraceae*, a bacterial family comprised of genera *Campylobacter*, *Arcobacter* and *Sulfurospirillum* (Vandamme, 2000). Among the 16 species and six subspecies of *Campylobacter*, two are most commonly isolated from stool samples of human gastroenteritis (Vandamme, 2000). They are *Campylobacter jejuni* subspecies *jejuni* and *Campylobacter coli*. *C. jejuni* accounts for approximately 95% of *Campylobacter* spp. caused human gastroenteritis, and *C. coli* are responsible for approximately 3 - 4% of the human illness.

Campylobacter spp. Are often a normal part of the intestinal flora of young cattle, sheep, goats, dogs, rabbits, monkeys, cats, chickens, turkeys, ducks, seagulls, pigeons, blackbirds, starlings and sparrows pigs (Nielsen *et al*., 1997; Smibert, 1984), and in blood and faecal material from humans with *Campylobacter* enteritis. They have also been found in the reproductive organs and oral cavity of humans and animals. Healthy puppies and kittens, rodents, beetles and houseflies have also been shown to carry *Campylobacter* spp. (Hartnett *et al*., 2002).

Growth characteristics

Campylobacter spp. require microaerophilic conditions for growth and have varying degrees of oxygen tolerance (3 - 5%) between species (Forsythe, 2000). Optimal growth occurs under conditions of 5% oxygen and 2 - 10% carbon dioxide (Park, 2002). Most strains do not grow in the presence of air, other than a few that may grow slightly under aerobic conditions. Some species can grow under anaerobic conditions with fumarate, formate and fumarate, or fumarate and hydrogen in the medium (Smibert, 1984; Vandamme, 2000).

Campylobacter spp. grow optimally at 42 -4 3^{o}C. *C. jejuni* can grow in the temperature range of 30 - 45^{o}C, pH of 4.9 - 9.5 and water activity above 0.99. At 32^{o}C, *C. jejuni* may double its biomass in approximately 6 hours (Forsythe, 2000). *Campylobacter* spp. do not multiply at temperatures below 30^{o}C (Park, 2002), which means that the numbers of *Campylobacter* spp. in foods will not increase at normal room temperatures (20 – 25^{o}C). Although unable to grow below 30^{o}C, *Campylobacter* spp. remain metabolically active, are able to generate ATP, and are motile at temperatures as low as 4^{o}C(Park, 2002).

Although *Campylobacter* spp. are considered thermotolerant, they are sensitive to heat and are readily inactivated by pasteurisation treatment or domestic cooking processes. Cooking at 55 - 60^{o}C for several minutes readily destroys *Campylobacter* spp. The D-value for *C. jejuni* at 50^{o}C is 0.88 - 1.63 minutes (Forsythe, 2000). *Campylobacter* spp. are also sensitive to freezing and/or freeze thawing.(Chan *et al*., 2001).

Other than temperature, a range of other environmental factors including desiccation, oxidation and osmotic stress influences the survival of *Campylobacter* spp. *Campylobacter* spp. are highly sensitive to desiccation and do not survive well on dry surfaces (Fernandez, 1985).

The microaerophilic nature of *Campylobacter* spp. means that these organisms are inherently sensitive to oxygen and its reduction substances (Park, 2002). *Campylobacter* spp. are much less tolerant to osmotic stress than a number of other foodborne pathogenic bacteria. For example, they are not capable of multiplication in an environment where sodium chloride concentration is 2% or higher (Doyle and Roman, 1982)

Due to its sensitivity to environmental conditions and inability of growth at temperatures below 30^{o}C or under aerobic conditions, the ability of *Campylobacter* spp. to multiply outside of an animal host is severely restricted. Although not capable of multiplication in food during processing or storage, *Campylobacter* spp. may have the ability to survive outside their optimal growth conditions (Park, 2002).

Pathology of illness

C. jejuni causes fever and enteritis in human, resulting in acute inflammatory diarrhoea with clinical signs similar to those of other acute bacterial infections of the intestinal tract, such as salmonellosis. Principal symptoms are diarrhoea, nausea, abdominal pain, fever, myalgia, headache, vomiting and blood in faeces (Lastovica and Skirrow, 2000).

The onset of symptoms is often abrupt with cramping abdominal pains quickly followed by diarrhoea. The mean incubation period is approximately 3 days with a range of 18 hours to 8 days. A particular feature of infection is abdominal pain, which may become continuous and sufficiently intense to mimic acute appendicitis. This is the most frequent reason for admission of *Campylobacter* enteritis patients to hospital (Skirrow and Blaser, 2000).

Although incidents are rare, *Campylobacter* spp. have been implicated in causing a range of extra-intestinal infections including appendicitis, haemolytic uraemic syndrome, abortion, hepatitis, cholecystitis, pancreatitis, nephritis and others (Skirrow and Blaser, 2000). *C. jejuni* may cause septicaemia, meningitis and serious neurological disorders such as Guillain-Barré syndrome , an acute neuromuscular paralysis, and reactive arthritis such as Reiter syndrome (Lastovica and Skirrow, 2000).

Mode of transmission

Friedmann et al. (2000) examined data from 111 food and waterborne outbreaks of *Campylobacter*iosis reported in the US between 1978 - 1996. Other than unknown foods, milk and water were the most common food vehicles associated with transmission of *Campylobacter* spp. Raw (unpasteurised) milk is largely responsible for dairy-related transmission. Of four milk-borne outbreaks in the period of 1990 - 1992, three were linked to raw cows' milk and raw goats' milk (CDC 2003). Surveys in other developed countries, including the United Kingdom, Sweden, Germany, New Zealand, Denmark, US and Norway, indicate milk is the most frequent cause of foodborne *Campylobacter* spp. infection (Friedman *et al*., 2000). Outbreak data of foodborne *Campylobacter*iosis recorded in

Australia between 1992 - 2001 present a similar picture to the above, where approximately 42% of recorded outbreaks were the result of consumption of milk, and among this, raw milk accounted for approximately 80% of milk-borne *Campylobacter* spp. outbreaks.

Published information (Eberhart-Phillips *et al*., 1997; Friedman *et al*., 2000; Vellinga and Loock, 2002; World Health Organisation, 2000) suggests that major routes of *Campylobacter* spp. transmission to humans are:

- Consumption of food contaminated with *Campylobacter* spp., including consumption of raw and unpasteurised milk and milk products, consumption of undercooked meat such as poultry meat and consumption of raw seafood
- Consumption of water contaminated with *Campylobacter* spp.
- Bathing or swimming in a *Campylobacter* spp. contaminated lake or pool
- Direct contact with infected farm animals, such as cattle, sheep, chicken, etc.
- Contact with infected domestic animals, such as a pet dogs, cattle and bird

Incidence of illness

C. jejuni is one of the most commonly reported aetiological agent of foodborne illness in developed countries, including Australia, NZ, UK and USA (Mead *et al*., 1999; Park, 2002). In the USA, approximately 80% of all the cases of human Campylobacteriosis are foodborne (Mead *et al*., 1999). In the period of 1998 – 2004, the notification rate of Campylobacteriosis in Australia has been 100 – 120 cases per 100,000 population (OzFoodNet, 2005). Notification rates were highest in the 0 – 4 year age group (OzFoodNet, 2005).

Occurrence in foods

Foods potentially contaminated with *Campylobacter* spp. include raw and unpasteurised milk and milk products, raw poultry, raw beef, raw pork and raw shellfish, as well as foods that may have been exposed to water contaminated with *Campylobacter* spp. (Institute of Food Technologists, 2002).

Virulence and infectivity of Campylobacter

Although not fully understood, *Campylobacter* spp. virulence is thought to involve production of microbial toxins. An enterotoxin (Wassenaar, 1997), abbreviated as CJT for *C. jejuni* toxin, is immunologically similar to the Vibrio cholerae toxin and the *E. coli* heat-liable toxin. At least six cytotoxins have been observed in *Campylobacter* spp., these being a 70-kDa cytotoxin, a Vero/HeLa cell cytotoxin, a cytolethal distending toxin (CDT), a shiga-like toxin, a haemolytic cytotoxin and a hepatotoxin. The CDT toxin has been shown to cause dramatic distension of human tumour epithelial cells, which leads to cell disintegration (Pickett *et al*., 1996). Active CDT toxin has been found in roughly 40% of the over 700 *Campylobacter* strains tested (Johnson and Lior, 1988). However, the role of enterotoxin and the cytotoxins in *Campylobacter* pathogenesis has not been fully identified.

Dose Response

Dose-response relationships have been developed based on results from human feeding studies, whereby human volunteers were fed known numbers of *Campylobacter* spp. cells and then monitored for their response (Black *et al*., 1988). These models make the assumption that (1) a single cell has the ability to initiate an infection and (2) the probability of causing infection increases as the level of the pathogen increases. Data from human trial experiments indicates that *Campylobacter* spp. infection correlates proportionally to the dose ingested and gradually reaches saturation. Despite a direct dose-response relationship being observed for the probability of infection, the probability of illness following from infection was independent of the dose ingested. The FAO/WHO Joint Expert Group on Microbiological Risk Assessment proposed a conditional probability of illness based on the probability of infection. Beta distribution of this conditional probability (Hartnett *et al*., 2002) suggests that the probability of illness is 20 - 50% after the establishment of an infection by *Campylobacter* spp.

For the human feeding trials 50% of individuals who ingested the minimum dose of 800 cells became infected (Black *et al*., 1988). Taking into consideration the limited size of the study, it has been proposed that the lowest infective dose would be somewhere close to 100 cells, which is comparable with epidemiological data (Prendergast *et al*., 2004)

Immune status

People with existing diseases are considered to have a higher susceptibility to *Campylobacter*iosis than the general population (Pigrau *et al*., 1997). The incidence of *Campylobacter* spp. infection in patients with AIDS has been calculated to be 40-fold higher than that in the general population (Sorvillo *et al*., 1991). In addition, 16% of *Campylobacter* spp. infections resulted in bacteraemia in these immunocompromised patients, a rate much higher than those occurring in the general population.

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2 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, non-spore forming rod-shaped bacteria that may be isolated from a variety of sources including soil, silage, sewage, food-processing environments, raw meats and the faeces of healthy humans and animals (FDA 2003). *L. monocytogenes* belongs to the genus *Listeria* along with *L. innocua, L. welshimeri, L. selligeri, L. ivanovii* and *L. grayi*. Thirteen serotypes are associated with *L. monocytogenes* (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7).

Growth characteristics

Growth of *L. monocytogenes* in foods is influenced by a variety of factors, including the nature and concentration of essential nutrients, pH, temperature, water activity, the presence of food additives that could enhance or inhibit growth and presence of other microbial flora (Lovett *et al*., 1987). Under conditions outside the growth range, the bacteria may survive and growth may recommence once suitable conditions are encountered. Temperatures of >50ºC are lethal to *L. monocytogenes*. When in a suitable medium, *L. monocytogenes* can grow between ~0 - 45°C. Although *L. monocytogenes* does not grow below –1.5ºC, it can readily survive at much lower temperatures. Nonetheless, freezing and frozen storage will cause a limited reduction in the viable population of *L. monocytogenes*. Optimal conditions for growth are between 30 - 37ºC (Ryser and Marth, 1999).

L. monocytogenes will grow in a broad pH range with the upper limit being approximately 9.3 and the lower limit being 4.6 - 5.0 (ICMSF, 1996). Although growth at pH <4.3 has not yet been documented, *L. monocytogenes* appears to be relatively acid tolerant. It has been suggested that food fermentations, which involve a gradual lowering of pH, could lead to acid adaptation of *L. monocytogenes*.

Like many bacterial species, *L. monocytogenes* grows optimally at a water activity (a_w) of approximately 0.97. However, when compared with most foodborne pathogens, the bacterium has the unique ability to multiply at water activity values as low as 0.90. While it does not appear to be able to grow below 0.90, the bacterium can survive for extended periods at lower values (Ryser and Marth, 1999).

L. monocytogenes is reasonably tolerant to salt and can grow in NaCl concentrations up to 10% (Sutherland *et al*., 2003). Extended survival occurs at a wide range of salt concentrations and *L. monocytogenes* has survived for up to eight weeks in a concentration of 20% NaCl (Sutherland *et al*., 2003). Survival in the presence of salt varies with storage temperature and studies have indicated that survival of *L. monocytogenes* in concentrated salt solutions can be increased dramatically by lowering the incubation temperature (Ryser and Marth, 1999). *L. monocytogenes* grows well under both aerobic and anaerobic conditions (Ryser and Marth, 1999; Sutherland *et al*., 2003).

The listericidal effect of preservatives is strongly influenced by the interactive effects of temperature, pH, type of acidulant, salt content, water activity, and type and concentration of food additives present in the food. For example, the ability of potassium sorbate to prevent growth of *L. monocytogenes* is related to temperature and pH. The lower the storage temperature and pH of the medium, the greater the effectiveness of sorbates against *L. monocytogenes*. Sodium benzoate is more inhibitory to *L. monocytogenes* than is either

potassium sorbate or sodium propionate. Inhibition and inactivation of *L. monocytogenes* in the presence of sodium benzoate is affected by temperature (more rapid at higher than lower incubation temperatures), concentration of benzoic acid (more rapid at higher than lower concentrations) and pH (more rapid at lower rather than higher pH values) as well as the type of acid used to adjust the growth medium (Ryser and Marth, 1999).

Pathology of illness

There are two main forms of illness associated with *L. monocytogenes* infection: *Listeria* gastroenteritis, where usually only mild symptoms are reported, and invasive listeriosis, where the bacteria penetrate the gastrointestinal tract and invade normally sterile sites within the body (FDA 2003).

Symptoms of the mild form of *L. monocytogenes* infection are primarily those generally associated with gastrointestinal illness: chills, diarrhoea, headache, abdominal pain and cramps, nausea, vomiting, fatigue, and myalgia (FDA 2003). The onset of illness is usually greater than 12 hours.

Invasive listeriosis is clinically defined when the organism is isolated from blood, cerebrospinal fluid or an otherwise normally sterile site (*e.g.* placenta, foetus). The manifestations include septicaemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion in the second or third trimester, or stillbirth (FDA 2003). The onset of these manifestations is usually preceded by influenza-like symptoms including persistent fever. Gastrointestinal symptoms such as nausea, vomiting and diarrhoea may also precede the serious forms of listeriosis. Listeriosis typically has a 2 - 3 week incubation time, but onset time may extend to 3 months (FDA/Centre for Food Safety and Applied Nutrition, 2003).

It is estimated that approximately 2 – 6% of the healthy human population harbour *L. monocytogenes* in their intestinal tract, which suggests that people are frequently exposed to *L. monocytogenes* (Farber and Peterkin, 1991; Rocourt and Bille, 1997). This may also suggest that most people have a tolerance to infection by *L. monocytogenes*, and given the relatively low number of reported cases, exposure rarely leads to serious illness in healthy individuals (Hitchins, 1996; Marth, 1988).

Mode of transmission

Foodborne exposure is the primary route of transmission for listeriosis, however listeriosis can be transmitted vertically (*i.e.* mother to child), zoonotically and through hospital acquired infections (Bell and Kyriakides, 2005; Ryser and Marth, 1999).

Incidence of illness

Most cases of listeriosis are sporadic. The number of reported cases of invasive listeriosis in Australia between 2001 - 2004 varied between 61 – 72 cases (OzFoodNet, 2002b; Anon, 2002; Anon, 2003a; Anon, 2004b), which equates to approximately 3 – 4 cases per million population per annum. In Australia, the exact mortality rate is not known, although the data

available would suggest a rate of approximately 23%. The case fatality rate in New Zealand is approximately 17% (Anon, 2004b).

The estimated incidence of invasive listeriosis in European countries has been reported to between 0.3 - 7.5 cases per million of the general population per annum (European Commission, 2003a). In France, the estimated incidence is sixteen cases per million (general population) per annum (Bille, 1990; ICMSF, 1996). The annual incidence of listeriosis in the US has been estimated to range from 3.4 per million (CDC 2002) to 4.4 per million (Tappero *et al*., 1995). Of all foodborne pathogens, *L. monocytogenes* results in the highest hospitalisation rate in the US, with fatality rates of 20 - 30% being common (WHO/FAO, 2004).

Outbreaks of invasive listeriosis have been linked to Hispanic-style soft cheeses; soft, semi-soft and mould-ripened cheeses; hot dogs; pork tongue jelly; processed meats; pate; salami; pasteurised chocolate flavoured milk; pasteurised and unpasteurised milk; butter; cooked shrimp; smoked salmon; maize and rice salad; maize and tuna salad; potato salad; raw vegetables; and coleslaw (FDA 2003). In addition, sporadic cases have been linked to the consumption of raw milk; unpasteurised ice cream; ricotta cheese; goat, sheep and Feta cheeses; soft, semi-soft and mould-ripened cheeses; Hispanic-style cheese; salami; hot dogs; salted mushrooms; smoked cod roe; smoked mussels; undercooked fish; pickled olives; raw vegetables; and coleslaw (WHO/FAO, 2004).

Occurrence in foods

L. monocytogenes has been found in foods such as milk, dairy products (particularly soft-ripened cheeses), meat, poultry, seafood and vegetables. The worldwide prevalence of *L. monocytogenes* in raw milk is estimated to be around 3 - 4% (Doores and Amelang, 1988; Hayes *et al*., 1986; Lovett *et al*., 1987). In Australian surveys on soft and surface ripened cheeses and ice-cream, *L. monocytogenes* has been isolated from 2% of locally produced cheese samples and 6% of ice-cream samples (Sutherland *et al*., 2003). For imported cheeses, Camembert and blue vein, 7% were positive for *L. monocytogenes* (Sutherland *et al*., 2003). For European soft and surface-ripened cheeses, 25% have been found to be positive for *L. monocytogenes* (Terplan, 1988).

Meat products from which *L. monocytogenes* has been isolated include beef, lamb, pork, minced meat products, sausages, salami, ham, mettwurst, pate, frankfurters and vacuumed packed meat, chicken products, and processed seafood (Cox *et al*., 1999; Farber and Peterkin, 1991; Ojeniyi *et al*., 2000). Additionally vegetable products have also been shown to be contaminated (Brackett, 1999; Heisick *et al*., 1989).

Virulence and infectivity of L. monocytogenes

When ingested, *L. monocytogenes* penetrates the intestinal tissue and is taken up by macrophages and non-phagocytic cells in the host. *L. monocytogenes* is disseminated throughout the host via blood or lymphatic circulation to various tissues. Its presence intra-cellularly in phagocytic cells permits access to the brain and probably transplacental migration to the foetus in pregnant women. The pathogenesis of *L. monocytogenes* relies on its ability to survive and multiply in phagocytic host cells. Not all strains appear to be equally

virulent. The 4b and occasionally 1/2a and 1/2b serovars account for most cases of human listeriosis (ICMSF, 1996). The virulence of *L. monocytogenes* is increased when the bacterium is grown at low rather than high temperatures. The possibility exists that cold storage may enhance the virulence of some *L. monocytogenes* strains isolated from refrigerated foods (Ryser and Marth, 1999).

Dose Response

Cases of non-invasive listeriosis (also referred to as febrile Listerial gastroenteritis) have been observed during outbreaks, involving symptoms such as diarrhoea, fever, headache and myalgia, generally following a short incubation period (WHO/FAO, 2004). Insufficient quantitative data is available to develop a dose-response model for this milder form of listeriosis, however, outbreak situations have generally involved the ingestion of high doses of *L. monocytogenes*.

The dose-response relationship for invasive listeriosis is highly dependent on a number of factors, such as the virulence characteristics of the organism, the number of cells ingested, the general health and immune status of the host, and the attributes of the food matrix that may alter the microbial or host status. WHO/FAO (2004) and FDA/FSIS (2003) developed separate dose-response models for both healthy and susceptible populations by combining data from surrogate animal models with epidemiological data. The Exponential dose-response model was used for both populations. This dose-response model has a single parameter, the *r*-value. The *r*-value is the probability that a person will become ill from the consumption of a single *L. monocytogenes* cell. For the healthy population (classified as "intermediate-age") the median r-value was estimated to be 2.37×10^{-14}. For more susceptible populations the median *r*-value was estimated to be 1.06×10^{-12}. A more recent assessment of US epidemiological data on invasive Listeriosis in susceptible sub-populations which included genetic information regarding different *L. monocytogenes* strains (lineages), determined average *r*-values of 1.31×10^{-8} for lineage I and 5.01×10^{-11} for lineage II (Chen *et al*., 2006). Further analysis of the epidemiological data by the *L. monocytogenes* ribotype found *r*-values as small as 6.29×10^{-3}. These results suggest that there are large differences in virulence between *L. monocytogenes* strains.

The infectious dose is unknown but it is believed to vary depending on the strain and susceptibility of the individual. There is a lack of information concerning the minimal infectious dose, although it is generally thought to be relatively high (>100 viable cells) (ICMSF, 1996). From cases contracted via raw or inadequately pasteurised milk, it is assumed that for susceptible individuals, ingestion of fewer than 1,000 organisms may cause disease (FDA/FSIS., 2003). It is thought the consumption of food with exceptionally high levels of *L. monocytogenes* ($>10^7$/g) is required to cause the mild gastrointestinal form of illness in healthy persons (Sutherland *et al*., 2003).

Host factors

Specific sub-populations at risk for invasive listeriosis include pregnant women and their foetuses, neonates, the elderly and persons with a compromised immune system, whose resistance to infection is lowered (*e.g.* transplant patients, patients on corticosteroid treatments, AIDS patients and alcoholics). Less frequently reporte:; diabetic, cirrhotic, asthmatic and ulcerative colitis patients are also at a higher risk (FDA 2003). Another

physiological parameter thought to be relevant to susceptibility is a reduced level of gastric acidity (WHO/FAO, 2004).

Food Matrix

To date, the properties of the food vehicle have been viewed as having little effect on the infective dose of *L. monocytogenes*. However, it is possible that food vehicles with high buffering capacity may protect the bacteria from inactivation by the pH of gastric acids in the stomach. In general, there are insufficient data available as to whether the food matrix affects the dose-response curve for *L. monocytogenes* (WHO/FAO, 2004).

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3 Pathogenic *Escherichia coli*

Escherichia coli are members of the family Enterobacteriaceae and are a common part of the normal intestinal flora of humans and other warm-blooded animals. The organisms are described as gram-negative, facultative anaerobic rod shaped bacteria (Desmarchelier and Fegan, 2003). Although most strains of *E. coli* are considered harmless, the species does contain certain strains that can cause severe illness in humans (Bell and Kyriakides, 1998). Strains of *E. coli* are differentiated serologically, based on O (somatic) and H (flagella) antigens (Lake *et al*., 2003).

Pathogenic *E. coli* are characterised into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle *et al*., 1997). These groups include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC). Many synonyms are used to describe EHEC, including Shiga toxin-producing *E. coli* (STEC), Shiga-like toxin-producing *E. coli* (SLTEC), and verocytotoxin-producing *E. coli* (VTEC).

E. coli O157:H7 is the best known and most widely studied serotype of *E. coli*. One of its natural habitats is the intestines of cattle, which creates the potential for contamination of milk and dairy products. In spite of this risk, milk and dairy products have only occasionally been implicated in outbreaks of *E. coli* O157:H7 food poisoning, and even more rarely does an outbreak involve a pasteurised product (Kirk and Rowe, 1999).

Growth characteristics

Growth and survival of pathogenic *E. coli* is dependent on the simultaneous effect of a number of environmental factors such as temperature, pH and water activity. In general, pathogenic *E. coli* strains behave similarly to non-pathogenic strains, however certain EHEC strains have been found to have a higher tolerance to acidic conditions than other groups of *E. coli* (Desmarchelier and Fegan, 2003).

The optimum temperature for growth of *E. coli* is 37°C, and it can grow within the range of 7 - 8°C to 46°C (ICMSF, 1996). Heat sensitivity of pathogenic *E. coli* is similar to that of other Gram-negative bacteria and is dependent on the pH, water activity and composition of the food (Bell and Kyriakides, 1998). Due largely to its importance as a cause of foodborne illness in the USA, most studies on the growth and/or survival of pathogenic *E. coli* have been undertaken with *E. coli* O157:H7 (an EHEC organism). Studies on the thermal sensitivity of *E. coli* O157:H7 have revealed that it is no more heat sensitive than *Salmonella* spp. (Doyle and Schoeni, 1984). Therefore, heating a product to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7.

Studies have demonstrated that some EHEC strains are acid-tolerant and can survive for at least five hours at pH 3.0 - 2.5 at 37°C (Benjamin and Datta, 1995). Stationary phase and starved pathogenic *E. coli* have been found to have an increased acid tolerance compared with exponential growth phase organisms (Arnold and Kaspar, 1995). Pathogenic *E. coli* may therefore be able to survive and/or grow in food products previously considered too acidic to support the survival of other foodborne pathogens. The effect of pH on *E. coli* survival is, however, dependent on the type of acid present. For example, *E. coli* O157:H7

can survive in a medium adjusted to pH 4.5 with hydrochloric acid but not when adjusted to the same pH with lactic acid (ICMSF, 1996).

The minimum water activity required for growth of pathogenic *E. coli* is 0.95, or approximately 8% sodium chloride (ICMSF, 1996). In sub-optimal temperature or pH conditions, the required for growth increases (Desmarchelier and Fegan, 2003).

Pathology of illness

EPEC causes illness primarily in infants and young children in developing countries. Symptoms include watery diarrhoea with fever, vomiting and abdominal pain. The diarrhoea is usually self-limiting and of short duration, but can become chronic (more than 14 days). EPEC is also recognised as a foodborne and waterborne pathogen of adults, where it causes severe watery diarrhoea (with mucus, but no blood) along with nausea, vomiting, abdominal cramps, fever, headache and chills. Duration of illness is typically less than three days (Dalton *et al*., 2004; Doyle and Padhye, 1989)

ETEC is another major cause of diarrhoea in infants and children in developing countries, as well as being recognised as the main cause of 'travellers diarrhoea' (Doyle and Padhye, 1989). Symptoms include watery diarrhoea, low-grade fever, abdominal cramps, malaise and nausea. In severe cases the illness resembles cholera, with severe 'rice-water' diarrhoea and associated dehydration. Duration of illness is from 3 - 21 days (Doyle and Padhye, 1989).

EIEC cause a dysenteric illness similar to shigellosis. Along with profuse diarrhoea, symptoms include chills, fever, headache, muscle pain and abdominal cramps. Onset of symptoms is usually rapid (<24 hours) and may last several weeks (Doyle and Padhye, 1989).

EHEC infection normally results in diarrhoea-like symptoms. Haemorrhagic colitis, an acute illness caused by EHEC organisms, is characterised by severe abdominal pain and diarrhoea. This diarrhoea is initially watery but becomes grossly bloody. Symptoms such as vomiting and low-grade fever may be experienced. The illness is usually self-limiting and lasts for an average of 8 days. The duration of the excretion of EHEC is about one week or less in adults, but it can be longer in children (ICMSF, 1996).

Complications resulting from EHEC infections vary. About 5% of haemorrhagic colitis victims may develop haemolytic uraemic syndrome (HUS) (European Commission, 2000). This involves the rupture of red blood cells (haemolysis), subsequent anaemia, low platelet count and kidney failure. The case-fatality rate of HUS has been reported to be 3 – 7% (Codex Alimentarius Commission, 2002). Shiga toxins produced by EHEC attack the lining of the blood vessels throughout the body, predominantly affecting the kidney. However other organs such as the brain, pancreas, gut, liver and heart are also affected and may result in further complications such as thrombotic thrombocytopenic purpura.

Table 1: Clinical, pathological and epidemiological characteristics of disease caused by the five principal pathotypes of *E. coli* (Robins-Brown, 1987)

Pathotype	Clinical symptoms	Intestinal pathology	Susceptible population
ETEC	Watery, cholera-like diarrhoea	No notable change	Children in developing countries; travellers to those countries
EIEC	Bacillary dysentery	Inflammation and disruption of the mucosa, mostly of the large intestine	All ages; more common in developing countries
EPEC	Non-specific gastroenteritis	Attaching-effacing lesions throughout the intestine	Children under 2 years of age in developing countries
EHEC	Bloody diarrhoea	"Haemorrhagic colitis"; attaching-effacing lesions confined to the large intestine; necrosis in severe cases	Children and the elderly in developed countries.
EAEC	Persistent diarrhoea	Inflammation, cytotoxic changes in enterocytes (data from experimental studies)	Children in developing countries; travellers to those countries

Mode of transmission

Pathogenic *E. coli* are transmitted by the faecal-oral route. Sources of transmission include person-to-person, foodborne, waterborne (drinking water and direct contact with faecal contaminated water) and direct contact with infected animals (ICMSF, 1996).

Incidence and outbreak data

Infection with pathogenic *E. coli* is a cause of significant morbidity and mortality worldwide. Outbreaks caused by EPEC, ETEC and EIEC occur infrequently in developed countries (ICMSF, 1996). In contrast, outbreaks caused by EHEC are more common, with a number of large foodborne outbreaks being reported in many countries, including Australia (Goldwater and Bettelheim, 1998). In developing countries, the incidence of EHEC infection is reported to be much lower than that of ETEC and EPEC infection (Nataro and Kaper, 1998).

EIEC stains have been isolated with low frequency from diarrhoeal cases in both industrialised and less developed countries (Nataro and Levine, 1994). Outbreaks have occurred in hospitals, on a cruise ship, and from contaminated water (Desmarchelier and Fegan, 2003).

ETEC stains are a major cause of diarrhoea in infants and young children in developing countries, particularly in the tropics, and are a leading cause of travellers' diarrhoea (Doyle and Padhye, 1989; Gross and Rowe, 1985; Nataro and Levine, 1994). Although uncommon, a number of foodborne outbreaks due to ETEC have occurred internationally (Olsvik *et al*., 1991). Mead *et al.*(1999) estimated that ETEC infection is responsible for approximately 0.4% of foodborne illnesses in the USA. In 1983 a multi-state ETEC outbreak occurred in the USA that was associated with consumption of imported Brie and Camembert cheese (Anon, 1984; MacDonald *et al*., 1985).

EPEC stains have caused infantile diarrhoea in hospitals and nurseries in the UK and the USA (Nataro and Levine, 1994; Robins-Brown, 1987). In developing countries, EPEC stains are still responsible for a high incidence of sporadic infant diarrhoea. Limited information is

available on foodborne outbreaks associated with EPEC. An outbreak of EPEC (serotype O111) occurred amongst people on a coach trip to France, although no specific food was identified. The infection was believed to have been the result of consuming food at a restaurant in northern France (Wight *et al*., 1997).

In the USA, consumption of undercooked hamburger meat has been an important cause of EHEC outbreaks (Nataro and Kaper, 1998). Since its identification as a human pathogen in 1982, and implication in a number of outbreaks in the USA, *E. coli* O157:H7 has become identified as the most predominant cause of EHEC related disease (FAO/WHO, 2000). It is estimated that 85% of EHEC infections in the USA are foodborne (Mead *et al*., 1999). A large multi-state *E. coli* O157:H7 outbreak involving consumption of contaminated hamburgers occurred in December 1992 – January 1993 with 732 cases identified, of which 195 were hospitalised and 4 died (Nataro and Kaper, 1998). Foodborne outbreaks of *E. coli* O157:H7 have also been associated with consumption of contaminated fresh produce. In the United States, outbreaks occurred in 1995 and 1996 (70 and 49 cases respectively), which were traced to consumption of lettuce (Tauxe, 1997). Studies have shown that *E. coli* O157:H7 can be transmitted to lettuce plant tissue from soil contaminated with manure and contaminated irrigation water (Solomon *et al*., 2002). Another large *E. coli* O157:H7 outbreak occurred in the US in 1996 which was linked to apple juice. Although the low pH of fruit juices will generally not allow the survival and growth of many Enterobacteriaceae, some strains of *E. coli* O157:H7 may survive due to their high acid-tolerance. In 2002, an outbreak of *E. coli* O157:H7 in Canada was attributed to the consumption of unpasteurised Gouda cheese (Honish *et al*., 2005).

Over 200 non-O157 STEC serotypes have been isolated from humans, with the World Health Organisation identifying O26, O103, O111 and O145 as the most important foodborne non-O157 serogroups worldwide (WHO, 1998). STEC has been a notifiable disease in most Australia States and Territories since August 1998 (Roche *et al*., 2001). During the period of 2001 – 2005, the notification rate for STEC (excluding HUS cases) in Australia has been 0.2 – 0.3 cases per 100,000 population per annum (OzFoodNet, 2002; The OzFoodNet Working Group, 2003; OzFoodNet, 2004; OzFoodNet, 2005). *E. coli* O157 has been the most commonly reported serotype. Significant variations in notifications exist between states and territories, and part of this variation is likely to be a result of different practices employed by pathology laboratories when screening faecal samples for toxin producing *E. coli* (The OzFoodNet Working Group, 2003).

A large EHEC outbreak occurred in South Australia during 1995, which resulted in approximately 200 cases of illness. Twenty-two people aged between 4 months and 12 years developed HUS and were hospitalised and a 4 year old child died. Investigations of the outbreak identified EHEC strain O111:NM (or strain O111:H-, NM for non-motile) as the principal cause of the outbreak. A locally produced uncooked, fermented mettwurst was identified as the vehicle for the pathogen. The product was found to contain a variety of EHEC strains in addition to O111 (Paton and Paton, 1998).

Occurrence in food

Humans appear to be the primary reservoir of EIEC, ETEC and EPEC organisms (Desmarchelier and Fegan, 2003). Therefore, contamination of food with these organisms is often due to human faecal contamination, either directly from an infected food handler or

indirectly via contaminated water. Very little information is available on the occurrence of these organisms in food. The detection of these organisms in food is difficult, requiring sophisticated methodology and therefore food is not routinely screened for these organisms.

In general, EPEC and ETEC organisms are more commonly isolated in foods from developing countries and their presence is associated with poor hygiene (Desmarchelier and Fegan, 2003). EPEC has been isolated from milk products in Iraq as well as from a variety of raw and cooked food in Malaysia (Abbar and Kaddar, 1991; Norazah *et al*., 1998). In Brazil, EPEC has been isolated from 21.1% of soft cheeses sampled (n=45) and has frequently been isolated from pasteurised milk (Araujo *et al*., 2002; Da Silva *et al*., 2001). EIEC has only sporadically been isolated from foods (Olsvik *et al*., 1991).

In addition to being a major cause of infantile diarrhoea in developing countries, ETEC organisms are a leading cause of traveller's diarrhoea, which has been linked to the consumption of contaminated food and water (Nataro and Kaper, 1998). ETEC has been isolated from Brazilian fish and shrimp which were harvested from waters contaminated with raw sewage (Teophilo *et al*., 2002). ETEC has also been detected in sauces at Mexican-style restaurants, and in chilli sauce sold by street vendors in Mexico (Adachi *et al*., 2002; Estrada-Garcia *et al*., 2002). In general, these sauces had been prepared and handled under poor hygienic conditions. The major reservoir of EHEC organisms appears to be the intestinal tract of ruminants, in particular cattle and sheep (Desmarchelier and Fegan, 2003).
E. coli O157:H7 and other EHEC species have been isolated from both healthy and diarrhoeic animals, and individual animals can carry more than one serotype (Anon, 1998). Foods derived from these animals may become contaminated via exposure to faecal material during processing.

Prevalence of STEC in raw milk has been determined in a limited number of studies. Caution must be exercised when comparing results between independent studies due to differences in sample size, stage of production where the samples were taken and different methodologies used to isolate the organisms. *E. coli* O157:H7 is the most widely studied EHEC serovar due to it being associated with a large number of outbreaks worldwide. In general, prevalence of STEC in raw milk is low. Adequate pasteurisation will ensure that STEC is inactivated. Very little information is available of the prevalence of EHEC organisms in food in Australia. Of the limited studies undertaken, the prevalence of *E. coli* O157:H7 in beef and sheep meat appears to be low, however, the prevalence of non-O157:H7 EHEC serotypes is unknown (Phillips *et al*., 2001a; Phillips *et al*., 2001b; Vanderlinde *et al*., 1998; Vanderlinde *et al*., 1999).

Virulence and infectivity

Clinical, pathological and epidemiological characteristics of disease caused by pathogenic *E. coli* vary between pathotypes and are discussed below.

EPEC have technically been defined as "diarrhoeagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related either to heat-labile enterotoxins or heat-stable enterotoxins or to Shigella-like invasiveness" (Edelman and Levine, 1983). EPEC cause characteristic attaching and effacing lesions in the intestine, similar to those produced by EHEC, but do not produce Shiga toxins. Attachment to the intestinal wall is mediated by a plasmid-encoded outer

membrane protein called the EPEC Adherence Factor in type I EPEC. However, pathogenicity is not strictly correlated to the presence of the EPEC Adherence Factor, indicating that other virulence factors are involved (ICMSF, 1996).

ETEC that survive passage through the stomach adhere to mucosal cells of the proximal small intestine and produce a heat-labile toxin and/or a heat-stable toxin. The heat-labile toxins are similar in structure and mode of action to cholera toxin, interfering with water and electrolyte movement across the intestinal epithelium (Desmarchelier and Fegan, 2003). If the volume of accumulated fluid exceeds the normal absorptive capacity of the large intestine, the excess is evacuated as watery diarrhoea.

EAEC strains are defined as *E. coli* strains that do not secrete heat-labile or heat-stable toxin. These strains adhere to cultured human epithelial cells in a characteristic aggregative or "stacked-brick" pattern (Yatsuyanagi *et al*., 2002). The mechanisms causing enteric disease are not fully understood, however EAEC have been associated with persistent diarrhoea, primarily in infants and children (Desmarchelier and Fegan, 2003).

Following ingestion, EIEC invade epithelial cells of the distal ileum and colon. The bacteria multiply within the cytoplasm of the cells, causing cell destruction and ulceration. Pathogenicity is associated with a plasmid-encoded type III secretory apparatus and other plasmid-encoded virulence factors (Desmarchelier and Fegan, 2003).

The Shiga toxins (Stx1 and Stx2) of EHEC are closely related, or identical, to the toxins produced by Shigella dysenteriae. Additional virulence factors allow the organism to attach tightly to intestinal epithelial cells, causing what is commonly referred to as attaching-and-effacing lesions.

Dose response

EPEC: It is thought that only a few EPEC cells are necessary to cause illness in children (FDA 2003). Volunteer studies in adults demonstrated that illness could be caused by ingesting $10^6 – 10^{10}$ cells with sodium bicarbonate to neutralise stomach acidity (Doyle and Padhye, 1989).

ETEC: Volunteer studies have shown that $10^8 – 10^{10}$ cells of ETEC are necessary for illness in adults (DuPont *et al*., 1971) although the infective dose is probably less for infants and children (FDA 2003).

EIEC: Volunteer studies have shown that 10^8 EIEC cells are necessary to cause illness in adults, with the infectious dose reduced to 10^6 when ingested with sodium bicarbonate (DuPont *et al*., 1971). However, the USA and Drug Administration (FDA) suggest that as few as 10 cells may be needed to cause illness in adults, based on the organisms similarity with Shigella (FDA 2003).

The dose-response relationship for EHEC is complicated by the large number of serotypes and the association of EHEC with a variety of foods. Haas *et al.* (2000) developed a dose-response relationship for *E. coli* O157:H7 based on data from a prior animal study undertaken by Pai *et al.* (1997) which involved oral administration of bacterial suspension to infant rabbits. The model was validated by comparison with two well-documented human

outbreaks, one foodborne and the other waterborne. The model estimated that the dose required to result in 50% of the exposed population to become ill was 5×10^5 organisms. The corresponding probability of illness for the ingestion of 100 organisms was 2.6×10^{-4}.

Dose-response relationships for *E. coli* O111 and O55 have been developed from human feeding trial data (Haas *et al*., 2000). The relationship estimated a dose required for 50% of the exposed population to become ill was 2.55×10^6 and the probability of illness for ingestion of 100 organisms was 3.5×10^{-4}. Investigations of other known outbreaks of foodborne illness due to *E. coli* O157:H7 and systematic studies aimed at quantifying the dose–response relationship suggest as few as 1 – 700 EHEC organisms can cause human illness (FDA 2003).

Host susceptibility

A variety of host factors may be important in the pathogenesis of specific *E. coli* serotypes. In general, the young and the elderly appear to be more susceptible to pathogenic *E. coli* infection. Epidemiological studies have identified that children are at higher risk of developing post-diarrhoeal HUS than other age groups (Cummings *et al*., 2002).

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4 *Salmonella* spp.

Salmonellosis is a leading cause of enteric illness, with symptoms ranging from mild gastroenteritis to systemic illness such as septicaemia and other longer-term conditions. A wide range of foods have been implicated in foodborne salmonellosis. However, as the disease is primarily zoonotic, foods of animal origin have been consistently implicated as the main sources of human salmonellosis. The genus *Salmonella* is currently divided into two species: *Salmonella enterica* (comprising six subspecies) and *Salmonella bongori* (Brenner *et al*., 2000). The subspecies of most concern in relation to food safety is S. enterica subsp. enterica, as over 99% of human pathogens belong to this subspecies (Bell and Kyriakides, 2002).

Over 1,400 *S.* enterica subsp. enterica serotypes are currently recognised, and all are regarded as capable of causing illness in humans(Brenner *et al*., 2000). The formal names to describe *Salmonella* serotypes are rather cumbersome, e.g. S. enterica subsp. enterica serotype Typhimurium (formerly *Salmonella* typhimurium). For practical reasons, the shortened versions of these names are commonly used, such as *Salmonella* Typhimurium. Some *Salmonella* serotypes are host-adapted to individual animal species. For example *S.* Typhi and *S.* Paratyphi are specifically associated with infections leading to severe illness in humans (Bell and Kyriakides, 2002).

Growth characteristics

*Salmonella*e have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates (Jay *et al*., 2003). The rate of growth and extent of survival of the organism in a particular environment is influenced by the simultaneous effect of a number of factors such as temperature, pH, and water activity. Being facultative anaerobic, *Salmonella*e also have the ability to grow in the absence of oxygen. Growth and survival is also influenced by the presence of inhibitors such as nitrite and short-chain fatty acids (Jay *et al*., 2003).

The growth of most *Salmonella*e is substantially reduced at temperatures <15°C and prevented at <7°C (ICMSF, 1996). Growth generally does not occur at temperatures >46.2°C. The optimum temperature for growth is 35 – 43°C. Freezing can be detrimental to *Salmonella* survival, although it does not guarantee destruction of the organism (ICMSF, 1996). There is an initial rapid decrease in the number of viable organisms at temperatures close to freezing point as a result of freezing damage. However, at lower temperatures (-17 to -20°C) there is a significantly less rapid decline in the number of viable organisms. *Salmonella* spp. have the ability to survive long periods of time at storage temperatures of < -20°C (Jay *et al*., 2003). Heat resistance of *Salmonella* spp. in food is dependent on the composition, nature of solutes, pH, and water activity of the food (Jay *et al*., 2003). In general, heat resistance increases as the water activity of the food decreases. A reduction in pH results in a reduction of heat resistance (ICMSF, 1996).

The minimum pH at which *Salmonella* spp. can grow is dependent on the temperature of incubation, the presence of salt and nitrite and the type of acid present. However, growth can usually occur between pH 3.8 – 9.5 (Jay *et al*., 2003). The optimum pH range for growth is 7.0 – 7.5. Volatile fatty acids are more bactericidal than acids such as lactic and citric acid.

Water activity has a significant effect on the growth of *Salmonella* spp., with the lower limit for growth being 0.94 (ICMSF, 1996) *Salmonella* spp. can survive for long periods of time in foods with a low water activity (such as black pepper, chocolate, gelatine). Exposure to environments of low water activitycan greatly increase the heat resistance of *Salmonella* spp.

Pathology of illness

Outcomes of exposure to *Salmonella* spp.can range from having no effect, to colonisation of the gastrointestinal tract without symptoms of illness (asymptomatic), or colonisation with the typical symptoms of acute gastroenteritis (FAO/WHO, 2002). Gastroenteritis symptoms may include abdominal pain, nausea, diarrhoea, mild fever, vomiting, headache and/or prostration, with clinical symptoms lasting 2 – 5 days. Most symptoms of salmonellosis are mild, and only a low proportion of cases within the community are reported to public health agencies (Mead *et al*., 1999). In a small number of cases, *Salmonella* spp. infection can lead to more severe invasive diseases characterised by septicaemia and sometimes death. In a study of 48,857 patients with gastroenteritis (of which 26,974 were salmonellosis), Helms *et al.* (2003) found an association with increased short-term (mortality within 30 days of infection) and long-term (mortality within a year of infection) risk of death compared with controls.

In cases of acute gastroenteritis, the incubation period is usually 12 - 72 hours (commonly 12 - 36 hours) and is largely dependent on the sensitivity of the host and size of the dose ingested (FAO/WHO, 2002; Hohmann, 2001). Illness is usually self-limiting, with patients fully recovering within one week, although in some severe cases of diarrhoea, significant dehydration can ensue which may require medical intervention such as intravenous fluid replacement. Septicaemia is caused when *Salmonella* spp. enters the bloodstream, with symptoms including high fever, pain in the thorax, chills, malaise and anorexia (FAO/WHO, 2002). Although uncommon, long-term effects or sequelae may occur including arthritis, appendicitis, cholecystitis, endocarditis, local abscesses, meningitis, osteomyelitis, osteoarthritis, pericarditis, peritonitis, pleurisy, pneumonia and urinary tract infection (ICMSF, 1996). At the onset of illness large numbers of *Salmonella* spp. are excreted in the faeces. Numbers decrease with time, but the median duration of excretion after acute non-typhoid salmonellosis has been estimated at five weeks, and approximately 1% of patients become chronic carriers (Jay *et al*., 2003).

Due to the general self-limiting nature of the disease, antibiotics are not usually recommended for healthy individuals suffering from mild to moderate *Salmonella* spp. gastroenteritis (Hohmann, 2001). Antibiotics should be used, however, for those who are severely ill and for patients with risk factors for extra intestinal spread of infection, after appropriate blood and faecal cultures are obtained.

Of recent concern worldwide is the emergence of multiple antibiotic resistant strains of *Salmonella* spp., an example being S. Typhimurium definitive phage type 104 (DT104). Multi-resistant S. Typhimurium DT104 is a significant human and animal pathogen, with high morbidity observed in cattle and poultry (Crerar *et al*., 1999). To date, this organism is not endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay *et al*., 2003). *S*. Typhimurium DT104 constitutes 8 – 9% of human *Salmonella* spp. isolates in the USA. Sporadic human cases are reported in Australia, although these are commonly acquired

overseas (Blumer *et al*., 2003). During 2001 an outbreak of S. Typhimurium DT104 occurred in Victoria and was linked to contaminated imported halva (a sesame seed product).

Mode of transmission

Salmonellae are transmitted by the faecal-oral route. Sources of transmission include person-to-person, foodborne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals.

Incidence and outbreak data

Salmonellosis is one of the most commonly reported enteric illnesses worldwide (FAO/WHO, 2002). Approximately 7,000 - 8,000 cases of salmonellosis per annum are formally notified to health authorities in Australia (Hall, 2003). Taking into account under-reporting it has been estimated (based on published rates of under-reporting) that 80,000 cases of foodborne salmonellosis occur annually (Hall, 2003). The salmonellosis notification rate in Australia for 2002 was 40.3 cases per 100,000 population. This varied from 24.8 cases per 100,000 population in Victoria to 166.7 cases per 100,000 population in the Northern Territory (Anon, 2003a). Children less than five years of age have by far the highest notification rate, with a rate of 210.6 cases per 100,000 population reported for 2002 (Yohannes *et al*., 2004). The higher rate of notified salmonellosis cases in this age group may reflect an increased susceptibility upon first exposure, but may also be a result of other factors such as an increased likelihood of exposure and increased likelihood to seek medical care and be tested.

Of the total number of *Salmonella* serovars reported to Australian health authorities during 2002, S. Typhimurium 135 was the most commonly reported. Distribution of *Salmonella* serovars varies geographically, with the most commonly reported serovars in Queensland, Tasmania and the Northern Territory being *S.* Virchow (10%), *S.* Mississippi (48%) and *S.* Ball (15%) respectively. Of the other States and Territories, S. Typhimurium was the most commonly reported serovar, representing 34% of cases in the Australian Capital Territory, 28% in New South Wales, 60% in South Australia, 66% in Victoria and 15% in Western Australia. Salmonellosis notifications in Australia fluctuate seasonally, from a low in August - September to a peak in January - March, with 36% of salmonellosis cases notified during this period (Yohannes *et al*., 2004).

It has been estimated that in the USA (Mead *et al*., 1999) and England and Wales (Adak *et al*., 2002), 95% and 91.6%, respectively of salmonellosis cases are foodborne. Other sources of infection may be via contaminated water, person-to-person transmission and direct contact with infected animals. Based on results from national and international epidemiological data (primarily outbreak investigations) a wide range of foods have been implicated in human salmonellosis. Foods of animal origin (*e.g.* meat, eggs, and dairy) are important sources of human salmonellosis.

Following notifications of salmonellosis to Australian health authorities, over 50 epidemiological investigations are initiated each year in an attempt to identify a common source of infection (Anon, 2003a). It is often difficult, however, to confirm a single food

commodity as a source due to the difficulty of investigating commonly consumed foods, conducting trace-back, and lack of systematically collected microbiological data from foods.

In a review of reported foodborne disease outbreaks in Australia during 1995 – 2000, meats (in particular poultry meat) were associated with 33% of identified salmonellosis outbreaks (Dalton *et al*., 2004). A large outbreak (consisting of 502 cases) of *S.* Typhimurium 135a occurred in 1999 and was associated with consumption of unpasteurised commercial orange juice (Roche *et al*., 2001). In 2001 a community-wide outbreak of *S.* Typhimurium 126 occurred in South Australia (OzFoodNet, 2002). A subsequent case-control study associated illness with the consumption of chicken meat. This link was corroborated with microbiological testing of raw poultry, and the likely source of contaminated products was traced to a single poultry processing facility.

Occurrence in food

The primary reservoir of *Salmonella* spp. is the intestinal tract of warm and cold-blooded vertebrates. Infected animals shed large numbers in their faeces, and this leads to contamination of the surrounding environment including soil, pasture, streams and lakes. *Salmonella* spp. have been isolated from a wide range of foods, particularly those of animal origin and those foods that have been subject to faecal contamination (ICMSF, 1996). Raw meat products (in particular poultry) have frequently been associated with the presence of *Salmonella* (Bryan and Doyle, 1995). *Salmonella* spp. positive animals at the time of slaughter may have high numbers of organisms in their intestines as well as on external surfaces (faecal contamination of hides, fleece, skin or feathers). Cross contamination during processing may also lead to increased prevalence of *Salmonella* spp. in finished products (Bryan and Doyle, 1995). Pasteurisation of dairy products effectively inactivates *Salmonella* spp., however contamination of milk has occurred due to improper pasteurisation and/or post-processing contamination (Jay *et al*., 2003).

Virulence and infectivity

Once ingested, *Salmonella* spp. must be able to overcome the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defence mechanisms to enable infection (Jay *et al*., 2003). *Salmonella* spp. possess a number of structural and physiological virulence factors enabling them to cause acute and chronic disease in humans.

Virulence of *Salmonella* spp. vary with the length and structure of the O side chains of lipopolysaccharide molecules at the surface of the cell. Resistance of *Salmonella* spp. to the lytic action of complement is directly related to the length of the O side chain (Jay *et al*., 2003). The presence of virulence plasmids has been associated with the ability to spread rapidly after colonisation and overwhelm the host immune response (D'Aoust, 1997). These virulence plasmids are large cytoplasmic DNA structures that replicate independently of the chromosomal DNA. Virulence plasmids are present in a limited number of *Salmonella* serovars and have been confirmed in S. Typhimurium, S. Dublin, S. Gallinarum, S. Pullorum, S. Enteritidis, S. Choleraesuis and S. Abortusovis. It is notable, however, that virulence plasmids are absent from S. Typhi, which is host-adapted and highly infectious.

Once attached to small intestine epithelial cells, the organism is drawn into the host cell in a vesicle (endosome) where it can multiply in the mildly acidic environment. Heat labile enterotoxin may be released during *Salmonella* spp. growth, resulting in the loss of intestinal fluids. This enterotoxin is closely related functionally, immunologically and genetically to cholera toxin and the heat labile toxin of pathogenic *E. coli* (Jay *et al*., 2003). Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage of the intestinal mucosal surface and general enteric symptoms and inflammation. For non-typhoidal *Salmonella* spp., infection is generally limited to a localised intestinal event.

Dose response

Human feeding trials for a range of *Salmonella* serovars were undertaken during the 1950's to determine the relationship between the dose of pathogen ingested and the response of the individual (McCullough and Eisele, 1951a; McCullough and Eisele, 1951b; McCullough and Eisele, 1951c; McCullough and Eisele, 1951d). The study population consisted of healthy males confined in an institutional setting who were fed known doses of an individual *Salmonella* serovar. Infection was confirmed by recovering the administered *Salmonella* serovar from faecal samples.

Fazil (1996) combined all the data from the feeding trials and found that a single beta-Poisson relationship could adequately describe the dose-response for all serovars. However, a number of limitations exist on the use of such feeding trial data. Firstly the use of healthy adult male volunteers could underestimate the pathogenicity to the overall population. In addition, volunteers were exposed to high doses of *Salmonella* spp., with the minimum dose being 10^4 cells.

In dose-response analysis, the critical region is the lower-dose region, as these are the doses that are most likely to exist in real food contamination events. This requires extrapolation of the model to doses much lower than those used in the human feeding trials. It must also be noted that the dose-response models are based on the risk of infection as an endpoint rather than illness, and therefore may introduce a level of conservatism into the dose-response relationship.

It has been shown through salmonellosis outbreak investigations, that doses resulting in illnesses (gastroenteritis) were often several orders of magnitude lower than the doses reported in the feeding trials (D'Aoust, 1994). Using a reasonably large data set, the FAO/WHO in 2002 developed a dose-response model based on actual outbreak data. Although not subject to some of the inherent flaws associated with using purely experimental data, the data used in this model have a certain degree of uncertainty, which required assumptions to be made. This uncertainty is primarily due to the uncontrolled settings under which the information and data were collected. It is often difficult to determine the actual dose ingested (based on the level of the organism in the food at the time of consumption and the amount of food consumed), as well as determining the actual number of people exposed or ill during the outbreak.

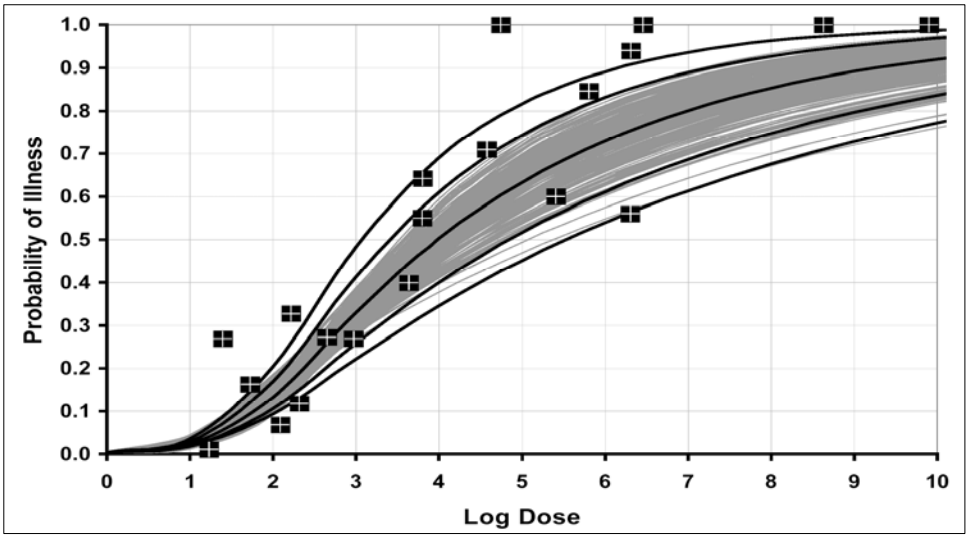

Figure 1: Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO, 2002).

Host factors

Individual susceptibility to *Salmonella* spp. infection and/or disease can vary significantly, depending on host factors such as pre-existing immunity, nutrition, age, ability to elicit an immune response, structural and functional anomalies of the intestinal tract, or pre-existing disease (Gerba *et al*., 1996; Jay *et al*., 2003). Individuals who are generally at greater risk of infection and/or risk of developing more severe outcomes from exposure to *Salmonella* spp. include the very young, the elderly, pregnant women and the immunocompromised (organ transplant patients, cancer patients and AIDS patients) (Gerba *et al.,* 1996).

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5 *Staphylococcus aureus*

The genus Staphylococcus is subdivided into 28 species and 8 subspecies. *Staphylococcus aureus* is a non-motile, Gram-positive, non-spore forming spherical bacterium. On microscopic examination, *S. aureus* appears in pairs, short chains, or bunched, grape-like clusters (Stewart, 2003). *S. aureus* is ubiquitous and inhabits the mucous membranes and skin of most warm-blooded animals, including all food animals and humans. Up to 50% of humans may carry this organism in their nasal passages and throats and on their hair and skin (FDA 2003).

S. aureus counts are often estimated by detecting coagulase-positive staphylococci, with further confirmatory tests required to specifically identify *S. aureus*. Nevertheless, the identification of coagulase-positive staphylococci or *S. aureus* is essentially an indicator test for the likelihood of enterotoxin production, as not all of these organisms have the ability to produce toxin, in addition, some strains of enterotoxin-producing staphylococci do not possess the coagulase enzyme (Stewart, 2003).

Growth characteristics

The temperature range for growth of *S. aureus* is 7 - 48°C with optimum growth occurring at 35 - 40°C. The temperature range for toxin production is 10 - 48°C with the optimum temperature being from 40 - 45°C. *S. aureus* grows over a wide water activity range (0.83 - 0.99) with an optimum aw of >0.99. The pH range for growth is 4.0-10 and the pH range for toxin production is 4.5 - 9.6 (ICMSF, 1996). *S. aureus* is tolerable to salt up to 25% NaCl (water activity of 0.85). *S. aureus* grows under both aerobic and anaerobic conditions; however growth is better in the presence of oxygen. Toxins are also produced under both aerobic and anaerobic conditions with greatest toxin production in the presence of oxygen (Bergdoll, 1989). *S. aureus* is generally considered a poor competitor with other bacteria.

S. aureus is readily killed at cooking and pasteurisation temperatures, however, heat resistance is increased in dry, high-fat and high-salt foods. In contrast, *S. aureus* enterotoxins are extremely resistant to heat. Heat resistance for enterotoxin B has been reported at D149=100 min (water activity of 0.99) (ESR, 2001). Heat resistances for *S. aureus* vegetative cells have been reported at D60 = 0.43 - 8.0 min whereas a time/temperature equivalent for enterotoxin is 121°C for 3 - 8 min (Baird-Parker, 1990; ICMSF, 1996). The enterotoxin is not affected by frozen storage.

Preservatives such as sorbate and benzoate are inhibitory to *S. aureus*, with their effectiveness increasing with a reduction in pH. Methyl and propyl parabens also have an effect on *S. aureus*, and high concentrations of carbon dioxide cause a substantial reduction in growth rates of *S. aureus* (Molin, 1985).

Most chemical sanitisers used routinely in the food industry such as chlorine, other halogens and quaternary ammonium compounds destroy *S. aureus* on surfaces. However some strains, for example those that become established on poultry processing equipment, have increased resistance (Bolton *et al*., 1988).

Pathology of illness

Staphylococcal foodborne illness is caused by the ingestion of food that contains preformed toxins produced by *S. aureus*. Usually this occurs when *S. aureus* is introduced into a food that will support growth of the organism, and that food is stored under conditions allowing the organism to grow and produce sufficient quantities of enterotoxin (Ash, 1997).

Symptoms generally appear around 3 hours after ingestion but can occur in as little as 1 hour (range 1 -6 hours) and are self-limiting (Ash, 1997; Stewart, 2003). Symptoms include nausea, vomiting, abdominal cramps of varying severity and diarrhoea. Some individuals may not demonstrate all of the symptoms associated with the illness. In severe cases, blood and mucus may be observed in stools and vomitus. Marked prostration, headaches and sweating accompany severe attacks and there may be fever or shock with subnormal temperatures and lowered blood pressure. Recovery is usually between 1 - 3 days requiring no medical treatment. Fatalities are rare, but are occasionally reported in young children and the elderly (Ash, 1997). All people are susceptible to staphylococcal food poisoning; however the intensity/severity may vary, depending on individual sensitivities.

S. aureus is also an opportunistic pathogen that causes infections via open wounds. *S. aureus* causes several types of infection including skin eruptions and inflammations (boils, acne, sties, etc) and wounds (Ash, 1997). *S. aureus* can also cause respiratory infections or may become established in the gut causing enteritis. *S. aureus* is an important bacterial cause of mastitis (an inflammatory disease of the mammary gland) in cows (Akineden *et al*., 2001). Mastitis in dairy cattle is characterised by changes in the udder tissue, clots and changes in milk quality, and is sometimes accompanied by heat and pain in the udder.

Mode of transmission

Staphylococcal food poisoning is caused by the consumption of food containing enterotoxins produced by certain strains of *S. aureus*. Despite wide-spread association of *S. aureus* with animals, humans tend to be the main reservoir for *S. aureus* infections in humans. Hand contact by food handlers with ready-to-eat foods is an important means by which *S. aureus* may enter the food supply. Foods that present the greatest risk of causing illness are those in which the normal flora has been destroyed (*e.g.* cooked meats) or inhibited (*e.g.* cured meats containing high salt content) (Stewart, 2003).

Incidence of illness

Food poisoning caused by *S. aureus* is one of the most common types of foodborne diseases world-wide (ICMSF, 1996). The incidence of staphylococcal food poisoning is often under-reported due largely to the self-limiting nature of illness, with most people recovering within 1 - 2 days without requiring medical attention. Foods commonly associated with staphylococcal food poisoning are meat and poultry, dairy products (particularly cheese and cream due to inappropriate handling, as well as contaminated raw milk), salads, cream filled bakery products and processed meat. Improper storage/ temperature abuse of food is the greatest factor attributing to outbreaks (Homberg and Blake, 1984).

In July 2000, an extremely large outbreak of staphylococcal food poisoning occurred in Japan, with an estimate 13,420 people being affected (Asao *et al*., 2003). The source of the outbreak was traced to powdered low-fat milk produced at a single factory in Osaka and was used as an ingredient in a number of dairy products. Staphylococcal enterotoxin was detected in the implicated milk powder; however, viable *S. aureus* was not isolated. This suggests that staphylococci were able to produce enterotoxin in the milk prior to pasteurisation, and remained immunologically and biologically active despite being pasteurised three times at 130°C for 2 – 4 seconds.

Despite *S. aureus* not being a notifiable illness in Australia, in 2002, three outbreaks of food poisoning attributed to *S. aureus* were reported. In one outbreak, a meal of lamb, rice and potatoes was implicated, in which Bacillus cereus was also identified. Other outbreaks implicated rice served in a childcare centre and pizza as the causative agent (Anon, 2003a; OzFoodNet, 2002). An outbreak was also reported in 2001 from consumption of barbequed chicken strongly suggesting an enterotoxin-producing bacterium as the causative agent, possibly *S. aureus* (Armstrong *et al*., 2002). In 2003, *S. aureus* was also implicated in foodborne illness after the consumption of a rice, beef and black bean sauce meal (Anon, 2003a).

Mead et al. (1999) stated that sporadic illness from *S. aureus* is not reportable in the US through either passive or active systems. The authors estimated 185,060 illnesses, 1753 hospitalisations and 2 deaths per year are attributed to *S. aureus* illness via contaminated food (Mead *et al*., 1999). Between 1975 and 1982, 36% of all reported *S. aureus* illness in the US were attributed to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and 1.4% attributed to milk products and seafoods. In 17.1% of cases the food involved was unknown (Genigeorgis, 1989).

In Canada, the average number of cases of illness from *S. aureus* for the years 1975 - 1984 was 232 cases per year (Todd, 1992). Foods implicated included pork (ham), turkey, chicken, cheese, pasta, salads and sandwiches. In France, *S. aureus* was attributed to 16 of 530 foodborne disease outbreaks recorded between 1999 - 2000 (Le Loir *et al*., 2003). Of these outbreaks, 32% were attributed tomilk products and especially cheeses, 22% were attributed to meats, 15% were attributed to sausages and pies, 11% were attributed to fish and seafood, 11% were attributed to eggs and egg products and 9.5% were attributed to poultry (Haeghebaert *et al*., 2002). In the UK for the years 1969 - 81, 1 - 6% of all cases of bacterial food poisoning were attributed to *S. aureus*. From 1982 - 1990, 0.5 - 1% of all cases of bacterial food poisoning was attributed to staphylococcal food poisoning. For the years 1969 - 1990 a study of 359 incidents of staphylococcal food poisoning was investigated. Poultry and poultry products accounted for 22% of incidents, most attributed to cold cooked chicken and in nine incidents turkey was the food vehicle (Bertolatti *et al*., 1996; Wieneke *et al*., 1993).

Occurrence in foods

Animals carry *S. aureus* on various parts of their bodies. Cow's udders and teats, and the tonsils and skin of pigs, chickens and turkeys are also known sources. Occurrence of staphylococci is common in raw milk. *S. aureus* in milk is related to the health status of the herd in respect to mastitis, and organisms numbers can range from <10 to several thousands per ml of milk with occasional counts of 105 cfu/ml (Asperger and Zangerl, 2002).

The prevalence of coagulase-positive staphylococci (which can include *S. aureus*, *S. intermedius* and some *S. hyicus*) in Australian beef and sheep carcasses and boneless beef and sheep surveyed in 1998 were 24.3% (beef carcasses), 24.1% (sheep carcasses), 17.5% (boneless beef) and 38.6% (boneless sheep) (Phillips *et al*., 2005; Phillips *et al*., 2001a; Phillips *et al*., 2001b).

Virulence and infectivity

S. aureus forms a wide range of substances associated with infectivity and illness, including the heat stable enterotoxins that cause food poisoning (Ash, 1997). Eleven antigenic types of staphylococcal enterotoxins are currently recognised, with types A and D being most commonly involved in food poisoning outbreaks. To date, staphylococcal enterotoxins A, B, C1, C2, C3, D, E, G, H, I and J toxins have been identified (Balaban and Rasooly, 2000). These enterotoxins are single-chain proteins comprising a polypeptide chain containing relatively large amounts of lysine, tyrosine and aspartic and glutamic acids and characterised by containing only two residues of half cystine and one or two residues of tryptophan. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity. They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They also resist chymotrypsine, rennin and papain (Bergdoll, 1989).

The production of enterotoxins is dependent on *de novo* synthesis within the cell. The quantity of toxin produced is variable and can be categorised by the type of toxin produced. Although weakly antigenic, enterotoxin antibodies have been produced in a variety of animal hosts. The mode of action of the toxin causing illness is not fully understood. However, it is thought that vomiting in response to ingestion of preformed toxin occurs due to the stimulation of local neuroreceptors in the intestinal tract, which transmit the stimuli to the vomiting centre of the brain via the vagus nerve and other parts of the sympathetic nervous system (ICMSF, 1996). A number of studies have identified toxin genes present in *S. aureus* isolates from the milk of cows with mastitis (Akineden *et al*., 2001; Cenci-Goga *et al*., 2003; Lim *et al*., 2004; Loncarevic *et al*., 2005; Zschock *et al*., 2004). The rate of enterotoxigenic *S. aureus* isolates from dairy cattle is highly variable and demonstrates the diversity of *S. aureus* strains (Cenci-Goga *et al*., 2003).

Dose response

The amount of enterotoxin that must be ingested to cause illness is not known exactly, but it is generally believed to be in the range 0.1 - 1.0 µg/kg (ICMSF, 1996). Toxin levels within this range are typically reached when *S. aureus* populations exceed 100,000/g (Ash, 1997).

Immune status

All people are believed to be susceptible to staphylococcal intoxication, but the severity of symptoms may vary depending on the amount of food ingested and the susceptibility of the individual to the toxin.

Food Matrix

The range of conditions that allow growth of staphylococci and the production of toxin vary with food type. The amount of starch and protein present in the food may enhance toxin production (Frazier and Westhoff, 1988).

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Appendix 15: Effect of thermisation and storage on the survival of microbial pathogens in cheese

The following questions were posed in respect to the effect of thermisation and storage on the survival of microbial pathogens in cheese:

Question 1
What is the impact of thermisation of milk (heat treatment at 62°C or above for 15 seconds or longer) on the identified microbial hazards?

(The main hazards for raw milk cheeses are: *Salmonella* spp., *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Campylobacter* spp., *Brucella*, and *Coxiella burnetii*)

Question 2
What is the impact of thermisation (heat treatment at 62°C or above for 15 seconds or longer) and storage condition for cheese or cheese products (at 2°C or higher for a period of 90 days from the date of manufacture) on the following identified microbial hazards?

(The main hazards for raw milk cheeses are: *Salmonella* spp., *L. monocytogenes, E. coli, S. aureus, Campylobacter* spp., *Brucella* spp., and *Coxiella burnetii*)

1 Introduction

Thermisation is a mild heat treatment of milk at 57 - 68°C for 10 - 20 seconds. As suggested by Van den Berg (1984), thermisation is not sufficient to reduce significantly the population of vegetative cells of the more heat resistance bacterial pathogens but creates a suitable environment for the multiplication of selected starter cultures in the manufacturing of various dairy products including cheeses.

Understandably, the above risk management question was raised against a processing requirement described in the Clause 2.1a of Standard 1.6.2 of the *Australia New Zealand Food Standards Code* (the Code) that applies to the processing of cheese and cheese products (Table 1).

Table 1: Clause 2.1a of Standard 1.6.2 of the Code

Cheese and cheese products must be manufactured from milk and milk products that have been heat treated –
(i) by being held at a temperature of no less than 72°C for a period of no less than 15 seconds, or by using a time and temperature combination providing an equivalent level of bacteria reduction; or
(ii) by being held at a temperature of no less than 62°C for a period of no less than 15 seconds, and the cheese or cheese product stored at a temperature of no less than 2°C for a period of 90 days from the date of manufacture.

Clause 2.1a (ii) involves two hurdles of sequential timing. The first is thermisation, *i.e.* heat treatment at 62°C or above with a heating period of 15 seconds or longer on milk or milk products used for cheesemaking. The second hurdle is storage/ripening of cheese at a temperature of 2°C or above for a period of 90 days from the date of manufacture of the

cheese. The combined effect of the two hurdles on microbial hazards is of an additive nature because of the sequential timing of the thermisation and storage/ripening. Clause 2.1a of Standard 1.6.2 might be taken to imply the combined effect of thermisation and storage/ripening on microbial pathogens in milk or milk products used for cheesemaking and on the cheese is equivalent to that achieved by standard conditions of pasteurisation of milk and milk products in cheesemaking, that is 72^{o}C for 15 seconds or 65^{o}C for 30 minutes.

To reflect such equivalence, the combined impact of thermisation (heat treatment at 62^{o}C or above for 15 seconds or longer) and storage/ripening (2^{o}C or above for a period of 90 days) on the identified microbial hazards in cheesemaking is described below, along with the impact of standard pasteurisation on the identified microbial hazards.

Other than to explore the vast number of possible time and temperature combinations, this assessment examines the impact of the minimum level of thermisation described in clause 2.1a of Standard 1.6.2 of the Code, *i.e.* 62^{o}C for a period of 15 second, and the minimum level of storage/ripening, *i.e.* 2^{o}C for a period of 90 days on the survival of the identified microbial pathogens. They are *Salmonella* spp.*, Listeria monocytogenes, E. coli, S. aureus, Campylobacter* spp.*, Brucella* spp., and *Coxiella burnetii*. Where the above temperature/time combination is lacking, the assessment examines the impact of the close proximities to the minima.

This assessment examines only the effect of thermisation and storage/ripening on microbial pathogens specified above in cheesemaking using raw milk or other no-pasteurised dairy ingredient. Cheesemaking using pasteurised milk and pasteurised dairy ingredients is considered elsewhere, asis the possible increase in the microbial population prior to storage/ripening in cheesemaking.

2 *Brucella* spp.

Effect of thermisation

Davis and Casey (1973) observed through a laboratory test tube method that treatment at 63.3^{o}C for 5, 10 or 15 seconds resulted in no inactivation of *Brucella abortus* in skimmed or whole milk that was either artificially inoculated at 1 - 4 x 10^{6} viable cells/ml or naturally infected at 1.25 x 10^{2} viable cells per ml. Treatment at 64.2^{o}C for 5, 10 or 15 seconds, however, resulted in partial inactivation of *B. abortus*.

A laboratory study of heat-resistance of eight *B. abortus* strains reported by Kronenwett *et al* (1954) found that the most heat-resistant strain of *B. abortus* (strain 2016) was inactivated at 62^{o}C in approximately 4 minutes. Z value in the temperature range of 61.5 - 67.8^{o}C varied from 4.3- 4.8^{o}C among the eight strains. The study concluded that thermal death times of the eight *B. abortus* strains were considerably below the pasteurisation time of 30 minutes at 63^{o}C. A safety margin of approximately 26 minutes was estimated at this temperature and time combination. A separate study (Van den Heever *et al.,* 1982) found that standard conditions of pasteurisation at either 65^{o}C for 30 minutes or 72^{o}C for 15 seconds resulted in no detectable *B. abortus* from a raw milk that was naturally contaminated by *B. abortus*.

The above data suggest that thermisation at 62^{o}C for 15 seconds alone has little or no killing effect on *B. abortus*.

Effect of storage

Gilman *et al*. (1946) reported that *B. abortus* survived for up to 6 months in cheeses that had been artificially inoculated at levels of approximately 1,000 cfu/ml and held at 4.4°C. Cheddar cheese made from milk that was naturally contaminated at levels of 700 - 800 cfu/ml was positive for culturable *B. abortus* for 3 months. Viable *B. abortus* were recovered from some, but not all of the cheeses tested after 6 months, but no viable *B. abortus* were detected after 1 year at 4.4°C. This study suggests that while storage/maturation at 2°C for 90 days is detrimental to the survival of *B. abortus*, complete inactivation of *B. abortus* is subject to the extent of the initial levels of contamination.

The combined effect of the minimum conditions of thermisation, *i.e.* 62°C for 15 seconds and storage/ripening, *i.e.* 2°C for 90 days, provides no guarantee to the elimination of *B. abortus* in cheese made with raw milk. Information on other *Brucella* species such as B. *melitensis* is lacking.

B. abortus was eliminated in Australia in 1989, and *B. suis* has been reported to be found in feral pigs in northern Australia according to World Organisation for Animal Health.

3 *Campylobacter jejuni* and *Campylobacter coli*

Effect of thermisation

Thermisation treatment at 62°C for 15 seconds can potentially result in 5 log reduction (5 D inactivation) of *C. jejuni* based on the D-value of 3 seconds at 62°C, extrapolated from the kinetics data of thermal inactivation studies of *C. jejuni* in milk (Waterman, 1982). On the other hand, only 3.5 D inactivation of *C. coli* in saline is expected under the same conditions of thermisation treatment based on an experimental D-value of 4.2 seconds at 62°C (Sorqvist, 1989).

Conclusions of several independent studies (D'Aoust *et al.,* 1988, Doyle and Roman, 1981; Waterman, 1982) suggest that standard conditions of pasteurisation at either 65°C for 30 minutes or 72°C for 15 seconds render milk free of *C. jejuni* and *C. coli*, where the initial concentration of *Campylobacter* spp. can be as high as 10^6 cfu/ml.

The above data suggests that unlike standard conditions of pasteurisation, thermisation at 62°C for 15 seconds can potentially lead to 3.5 to 5 D inactivation of *Campylobacter* spp. in milk. Dependent on the initial levels of contamination, thermisation at 62°C for 15 seconds alone may leave a residual level of *Campylobacter* spp. in the milk.

Effect of storage

Campylobacter spp. are microaerophilic, unable to grow at temperatures below 30°C (Park, 2002), not capable of multiplication in an environment where sodium chloride concentration is 2% or higher (Doyle and Roman, 1982), and have an optimal growth temperature at 42 - 43°C. Therefore storage/ripening of cheese at 2°C for 90 days is unlikely to result in an increase in, rather a decline of the population of *Campylobacter* spp. Doyle and Roman (1982) found that *Campylobacter* spp. could not survive long in raw milk because of the increase in milk aerobic plate count and a decrease in milk pH.

On temperature alone, *Campylobacter* spp. are more likely to die-off slowly under 2ºC than under a higher temperature (Blaser *et al.,* 1980).

Ehlers *et al.* (1982) in their study of the survival of *C. jejuni* in Cheddar and cottage cheese found *C. jejuni* died off quickly in the first 2 weeks of storage/ripening at 7^{o}C from an initial concentration of up to 10^{6} cfu/ml. No *C. jejuni* was detectable from the cheese after 30 days of storage. The authors suggested that a combination of low pH, low water activity and the presence of salt were among the reasons for the destruction of *C. jejuni* during the storage/ripening of the Cheddar cheese.

Based on the above, it is concluded that the population of *Campylobacter* spp. will decline rapidly upon thermisation at 62^{o}C for 15 seconds. At the end of storage/ripening at 2^{o}C for 90 days, detection of viable *Campylobacter* spp. in cheese is unlikely.

4 *Coxiella burnetii*

Effect of thermisation

In defining the optimal temperature and time combination to inactivate *Coxiella burnetii* present in raw milk, Enright *et al.* (1956) showed that heat treatment at 61.8^{o}C for 30 minutes was not adequate to eliminate *C. burnetii* from raw milk. Elimination of *C. burnetii* from raw milk, however, is ensured when the temperature is raised to 62.8^{o}C.

Elimination of *C. burnetii* from dairy products with higher levels of solids and fat requires further increase in pasteurisation temperature and/or time. For example, elimination of *C. burnetii* from cream which contains up to 40% butter fat, and chocolate milk which contains 4% butter fat and 22.5% total solids, requires pasteurisation temperatures/time to be raised to 65.6^{o}C for 30 minutes or 74.4^{o}C for 15 seconds. Elimination of *C. burnetii* from ice cream mix that contains up to 18% butter fat and 43% total solids requires pasteurisation temperature to be raised to 68.3^{o}C for 30 minutes or 79.4^{o}C for 25 seconds (Enright, 1961).

The above data suggest that under standard pasteurisation conditions (*i.e.* 65^{o}C for 30 minutes or 72^{o}C for 15 seconds), *C. burnetii* in milk is destroyed completely. However,minimum thermisation at 62^{o}C for 15 seconds would be inadequate to eliminate *C. burnetii* in milk.

Effect of storage

Data on the ability of growth or survival of *C. burnetii* during cheese storage/ripening is lacking because *C. burnetii* is an obligate organism and unable to grow outside of its host. Human infection by *C. burnetii* through consumption of unpasteurised milk is rare according to Tissot-Dupont *et al.* (2004).

Available information suggests that *C. burnetii* is likely to survive the minimum thermisation at 62^{o}C for 15 seconds. The effect of storage/ripening in cheesemaking at 2^{o}C for 90 on *C. burnetii* is not known due to lack of information.

5 Pathogenic *Escherichia coli*, primarily *E. coli* O157:H7

Effect of thermisation

Different strains of *E. coli* exhibit different sensitivities to heat treatment in milk processing. With three *E. coli* strains tested, Singh and Ranganthan (1980) found a pathogenic strain, O111:B4, was more sensitive to heat treatment than the two non-pathogenic strains. Strain O111:B4 has a z-value of approximately 5^{o}C in the temperature range of 50 to 63^{o}C while the non-pathogenic strains had a z-value of approximately 10^{o}C.

D'Aoust *et al.* (1988) demonstrated that with a mix of 10 strains of *E. coli* O157:H7 in milk at an initial concentration of approximately 2 x 10^5 cells/ml, heat treatment at 64.5, 66.0, or 72.0^{o}C for 16.2 seconds completely inactivated all *E. coli* O157:H7 cells. However, heat treatment at 60.0^{o}C and 63.0^{o}C for 16.2 seconds left a residual population of up to 2.3 x 10^4 cfu/ml and 9.3 x 10^3 cfu/ml, respectively.

Morgan *et al.* (1988) reported an estimated D-value of 7.7 - 14.4 seconds (depends on the recovery medium used) at 62.0^{o}C for a pathogenic *E. coli* strain in human milk. Based on this D-value, thermisation treatment at 62.0^{o}C for 15 seconds could inactivate up to 2 log of the *E. coli* population. Clementi *et al.* (1995) observed similar D-values of 4.8 seconds at 61.0^{o}C and 1.4 seconds at 63.3^{o}C for a pathogenic *E. coli* strain together with a z-value of 4.7^{o}C in the temperature range of 56.5 - 64.5^{o}C.

Thermisation at 64.5^{o}C for 17.5 seconds can result in 5 log reduction of *E. coli* O157:H7 in milk according to Schlesser *et al.* (2006). Pathogenic *E. coli* is completely destroyed by either batch pasteurisation at 65^{o}C for 30 minutes or high temperature short time pasteurisation at 72^{o}C for 15 seconds with a wide margin of safety (D'Aoust *et al.,* 1988; Clementi *et al.,* 1995; Hassan and Frank, 2000; Morgan *et al*, 1988; and Singh and Ranganthan, 1980).

The above data suggest that thermisation treatment at 62^{o}C for 15 seconds is highly detrimental to the survival of pathogenic *E. coli* in milk, potentially leads to up to 2 log reduction of the *E. coli* population.

Effect of storage

Schlesser *et al.* (2006) found that storage/ripening of Cheddar cheese made with raw milk at 7^{o}C for 90 days resulted in a reduction of the *E. coli* O157:H7 population by 2.4 - 3.5 x 10cfuml. The study also found *E. coli* O157:H7 level increased from an initial 3.3 x 10 - 4.8 x 10^3 cfu/ml prior to the storage/ripening in cheesemaking. Demonstrated by Ramsaran *et al.* (1988), the population of *E. coli* O157:H7 increased from the initial concentration of approximately 4.0 log (inoculated into the raw milk) to about 5.3 log at 75 days from the date of manufacture of a Feta cheese. The same study found that the population of
E. coli O157:H7 increased from the initial concentration of approximately 4.7 log to about 5.5 log at 65 days from the date of manufacture of a Camembert cheese.

The above information suggests that thermisation treatment at 62^{o}C for 15 seconds and subsequent storage/ripening at 2^{o}C for 90 days are more likely to result in approximately 3 log reductions of *E. coli* in cheesemaking. The initial level of *E. coli* contamination in the

raw milk and the chemical characteristics of the cheese itself determine the residual level of pathogenic *E. coli* in the final cheese.

6 *Listeria monocytogenes*

Effect of thermisation

A review of heat resistance data of *L. monocytogenes* in milk published prior to 1989 (Mackey and Bratchell, 1989) found *L. monocytogenes* was more heat resistant than most of the *Salmonella* serotypes. From over 30 sets of published heat resistance studies, Mackey and Bratchell (1989) reported a D-value of 131 seconds for *L. monocytogenes* at 60°C and a D-value of 42 seconds at 63°C. Data summarised by Doyle *et al.* (2001) showed a D-value of 20 - 46 seconds for *L. monocytogenes* at 63/63.3°C in raw milk, and 21 - 60 seconds at 62.7/62.8°C in sterile milk.

L. monocytogenes with an initial concentration at around 10^5/ml, survived heat treatment for 17.6 seconds at 60, 63, 64.5, 66.0 and 67.5°C, but not at 69 or 72°C (Farber *et al.,* 1988a).

Pasteurisation at 65°C for 30 minutes or 72°C for 15 seconds completely inactivated *L. monocytogenes* in raw milk according to Doyle *et al.* (2001) and Piyasena *et al.* (1998) at an initial concentration of up to 10^5 cfu/ml.

Subject to the initial level of *L. monocytogenes* in raw milk, the above data suggest that while standard pasteurisation conditions eliminate *L. monocytogenes* in cheesemaking, minimum thermisation at 62°C for 15 seconds appears inadequate for the same purpose.

Effect of storage

Ryser and Marth (1987b) demonstrated that *L. monocytogenes* could persist for up to 434 days post processing in artificially contaminated Cheddar cheese. The extent of inactivation of *L. monocytogenes* during cheese storage/ripening is dependent on the chemical characteristics of the cheese produced. As shown by Bachman and Spahr (1995), inoculated *L. monocytogenes* survived storage/ripening for 90 days at 11 - 13°C in a semi-hard Swiss cheese, but not in hard Swiss cheese. In addition, there is evidence to suggest that *L. monocytogenes* is capable of growth in soft cheeses, such as Camembert Cheese and blue cheese (Ryser and Marth, 1987a, Ramsaran *et al.,* 1988 and Papageorgiou and Marth, 1989b).

Based on the above data, it is predicted that *L. monocytogenes* is likely to survive the combined treatment of thermisation at 62°C for 15 seconds and the storage/ripening at 2°C for 90 days in at least some of the cheeses, for example, in soft Camembert cheese.

7 *Salmonella* spp.

Effect of thermisation

Doyle and Mazzotta (2000) found that a number of *Salmonella* serotypes including the most heat resistant serotype, *Salmonella* Senftenberg 775W, could be destroyed completely under the standard pasteurisation conditions, *i.e.* at 65°C for 30 minutes or at 72°C for 15 seconds. Serotype 775W is approximately twice more heat resistant than a mixture of human isolates

of *Salmonella* including, Typhimurium, Infantis, Hadar, Agona, Enteritidis, Heidelberg, Newport, Saint-paul, Thompson and Schwarzengrund according to D'Aoust et al. (1987). Thermisation treatment at 63°C for 17.6 seconds resulted in approximately 4 log reductions of *Salmonellae* in fluid milk, and at 60°C for 17.6 seconds approximately 2 log reductions of *Salmonellae* (D'Aoust *et al.,* 1987). A D-value of 6.6 seconds for *Salmonella* Typhimurium in milk at 62.8°C was shown by Bradshaw *et al*. (1987).

These heat inactivation parameters suggest that thermisation at 62°C for 15 seconds could potentially deliver approximately 2 log reductions of salmonellae in raw milk.

Effect of storage

Outbreak investigations in Ontario, Canada from 1980 to 1982, found viable *Salmonella* Muenster in raw milk Cheddar cheese after 125 days of storage/ripening at 5°C (Johnson *et al.,* 1990c). *Salmonellae* have been shown to be capable of survival for up to 8 months in naturally contaminated Cheddar cheese made of thermised milk. The storage/ripening temperature was at 5°C (D'Aoust *et al.,* 1985). White and Custer (1976) reported that *Salmonellae* (*S*. Newport, *S*. Newbrunswisk and *S*. Infantis) inoculated at 10^5 cfu/ml survived for 9 months in 1/3 of the Cheddar cheeses stored/ripened at 4.5°C and in 1/8 of the Cheddar cheese stored/ripened at 10°C. An earlier study showed *Salmonellae* survived in Cheddar cheese stored/ripened for up to 7 months at 13°C and for 10 months at 7°C (Park *et al.,* 1970).

The above data suggests that while approximately 2 log reductions of *Salmonellae* is likely to be achieved by thermisation at 62°C for 15 seconds, there is no guarantee that the subsequent storage/ripening for 90 days at 2°C will eliminate *Salmonellae* from cheeses made with raw milk. The quality of the raw milk, *i.e.* the initial level of *Salmonellae* contamination, ability of *Salmonella* to survive in cheese for a long period of time, and the varied characteristics of different cheeses all play a role with this uncertainty.

8 *Staphylococcus aureus*

Effect of thermisation

Demonstrated by several independent studies, standard pasteurisation at 65°C for 30 minutes or 72°C for 15 seconds completely inactivates *Staphylococcus aureus* in milk even at the level of 10^9 cfu/ml prior to pasteurisation (Firstenberg-Eden *et al.,* 1977; Parente and Mazzatura, 1991; Thomas *et al.,* 1966). D-value reported in these studies varied from 1.8, 3 and 10.2 seconds at 65°C, and 1.5 second at 72°C.

D-value of *S. aureus* in milk at 62°C ranged from 12 seconds (Walker and Harmon, 1966) to 27 seconds (Firstenberg-Eden *et al.,* 1977).

These data suggest that thermisation at 62°C for 15 seconds is likely to deliver approximately 1 log reduction of *S. aureus*, but cannot ensure complete inactivation of *S. aureus* in milk. At this time and temperature combination, enterotoxins produced by *S. aureus*, if already present in milk, will not be inactivated because a temperature of 121°C for 3 - 8 minutes treatment is required to inactivate 90% of the *S. aureus* enterotoxins produced according to Stewart (2003).

Effect of storage

Bachmann and Spahr (1995) demonstrated that *S. aureus* inoculated at approximately 5×10^5 cfu/ml, died off completely in hard Swiss cheese made of raw milk a day into the storage/ripening stage (at 11 - 13 ^{o} C). *S. aureus* survived for more than 60 days storage/ripening at 11 - 13^{o}C in semi-hard Swiss-type cheese made of raw milk, but no viable cells were detectable at 90 days of storage/ripening.

The above data suggests that approximately 1 log reductions of *S. aureus* is achievable by thermisation at 62^{o}C for 15 seconds. Subsequent storage/ripening at 2^{o}C for 90 days are likely to result in complete inactivation of *S. aureus* in hard Swiss-type cheeses and semi-hard Swiss cheese. Complete inactivation of *S. aureus* in other cheeses may not be achievable under the above conditions. In this case, residual level of *S. aureus* in the final cheese is likely to vary according to the chemical characteristics of the cheese.

9 Summary

Table 1 summarises the effect of thermisation of raw milk or dairy ingredients used for cheesemaking, *i.e.* heat treatment at 62^{o}C for 15 seconds and the subsequent storage/ripening of the cheese at 2^{o}C for 90 days on the following pathogenic bacteria, *Brucella* spp., *C. jejuni* and *C. coli*, *Coxiella burnetii*, pathogenic *E. coli* including *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp. and *S. aureus*.

C. jejuni and *C. coli* are of little concern as possible cause of human illness in thermised and ripened cheese made of raw milk. *Brucella* spp. is of less concern for domestically produced cheese made of raw milk because *B. abortus* has been eliminated in Australia and *B. suis* has been reported only in feral pigs in northern Australia. Little is known about the effect of storage/ripening on *C. burnetii* because of the inability of this organism to grow outside of its host. Although *S. aureus* is largely inactivated by the combined thermisation and storage/ripening in hard and semi-hard Swiss-type cheeses, little is known about its fate in other cheese varieties. Available scientific evidence suggests that *Salmonella* spp., *L. monocytogenes* and pathogenic *E. coli* are likely to survive the minima of the thermisation and storage/ripening conditions described above.

The final pH, water activity and salt content of the cheese exhibit significant influence on the extent of pathogen inactivation in cheesemaking (El-Gazzar and Marth, 1992; Hudson *et al.,* 2003). No attempt in the above assessment has been made with reference to the chemical properties of different cheeses including pH, water activity, salt content, milk fat content and dry matter because of the vast number of cheese varieties and vast range of chemical properties of different cheeses. As each cheese variety possesses its own unique set of chemical properties, it is unlikely that a generic requirement on thermisation and storage/ripening parameters could be prescribed, without being unreasonably restrictive, that will suit all cheese varieties made with raw milk.

The wide range of variations of prescribed minimum storage/ripening period for cheeses made of raw milk (without standard pasteurisation treatment) shown in Table 2 indicates that establishing minimum storage/ripening parameters for cheeses made of raw milk must take into consideration specific chemical characteristics of the each cheese variety. This is the same with minimum thermisation conditions. The decision making on the minimum

thermisation and storage/ripening parameters of any specific cheese or cheese product made with raw milk should preferably be based upon challenge studies of all the common pathogenic microorganisms likely to be encountered in the raw milk. In addition, a set of microbiological quality parameters for the raw milk used in making the cheese must be established as part of the criteria in making safe raw milk cheese. In some circumstances, some of the cheeses can only be made microbiologically safe with pasteurised dairy ingredients.

A report prepared by Institute of Environmental Science and Research Limited of New Zealand on thermisation in 2001 (Baldwin 2001) concluded that provided that the milk used is of good quality, the thermisation process and subsequent storage/ripening will result in the production of hard cheese that is safe for consumption, but not soft and fresh cheese. Here the thermisation process refers specifically to heat treatment at 64.5^{o}C for not less than 16 seconds. The storage/ripening condition refers specifically to 90 days or more from the date of commencement of manufacture at a temperature of 7^{o}C or higher. Hudson *et al*. (2003) concluded that storage/ripening appears to be an unreliable means of ensuring the safety of cheese made of raw milk. Although some cheeses may have characteristics that produce a high degree of inactivation of pathogens, such as the very hard Swiss-type cheese Emmentaler, Gruyere or Sbrinz, the effect of storage/ripening on the inactivation of microbial pathogens needs to be determined on a case by case basis.

Table 1: Summary of the effect of the minimum thermisation (62°C for 15 seconds) and minimum storage/ripening (2°C for 90 days) on pathogenic organisms discussed in this assessment

Organism	Effect of thermisation	Effect of storage/ripening	References
Brucella spp.	Little or no killing effect on *B. abortus* D_{62} = 4 min Z (61.5 – 67.8°C) = 4.3 – 4.8°C		Davis and Casey, 1973 Kronenwett *et al.,* 1954
		Viable *B. abortus* detectable for up to 6 months in Cheddar cheese stored at 4°C (initially inoculated at 10^3 cfu/ml)	Gilman *et al.,* 1946
C. jejuni and *C. coli*	3.5 to 5 log reduction D_{62} = 3 seconds for *C. jejuni* D_{62} = 4.2 seconds for *C. coli*		Sorqvist, 1989 Waterman, 1982
		Eliminated No C. jejuni was detectable after 30 days in Cheddar and cottage cheese, initial concentration at 10^6 cfu/ml and storage/ripening at 7°C	Ehlers *et al.,* 1982
Coxiella burnetii	Viable cells detected after heat treatment at 61.8°C for 30 min, but undetectable at 62.8°C for 30 min		Enright *et al.,* 1956
		Lack of information	
Pathogenic *E. coli* including *E. coli* O157:H7	Approximately 2 log reduction D_{61} = 4.8 seconds D_{62} = 7.7 to 14.4 seconds $D_{63.3}$ = 1.4 second Z (50 – 63°C) = 5 to 10°C Z (56.5 – 64.5°C) = 4.7°C		Clementi *et al.,* 1995 Morgan *et al.,* 1988 Singh and Ranganthan, 1980
		Approximately 1 log reduction *E. coli* O157:H7 reduced by 2.4 – 3.5 x 10^1 cfu/ml when stored/ripened at 7°C for 90 days	Schlesser *et al.,* 2006
L. monocytogenes	Incomplete inactivation D_{60} = 131 seconds $D_{62.7}$ = 60 seconds D_{63} = 42-46 seconds		Mackey and Bratchell, 1989 Doyle *et al.,* 2001 Farber *et al.,* 1988b
		L. monocytogenes survived for up to 434 days in Cheddar cheese; and for more than 90 days in semi-hard Swiss cheese stored/ripened at 11 to 13°C	Bachman and Spahr, 1995 Ryser and Marth, 1987b
Salmonella spp.	Approximately 2 log reduction $D_{62.8}$ = 6.6 seconds		D'Aoust *et al.,* 1987 Bradshaw *et al.,* 1987
		Viable *Salmonella* detected after 125 days and up to 8 month in Cheddar cheese stored/ripened at 5°C; for 9 months at 4.5°C and 10°C; for 7 months at 13°C and for 10 months at 7°C.	D'Aoust *et al.,* 1985 Johnson *et al.,* 1990c Park *et al.,* 1970 White and Custer, 1976
S. aureus	Approximately 1 log reduction D_{62} = 12-27 seconds D_{65} = 1.8-10.2 seconds D_{72} = 1.5 seconds		Firstenberg-Eden *et al.,* 1977 Parente and Mazzatura, 1991, Thomas *et al.,* 1966 Walker and Harmon, 1966
		Largely inactivated in semi-hard Swiss cheese *S. aureus* with initial concentration at 5 x 10^5 cfu/ml survived for more than 60 days but not 90 days in semi-hard Swiss-type cheese	Bachmann and Spahr, 1995

Table 2: Specific requirements for cheese products (latest revision, 1993) - Code of Federal Regulations (21 CFR part 133), The United States of America, 1 April 2005

Cheese	Moisture (w/w)	Minimum storage period	Milk fat content (w/w)
Asiago fresh and Asiago soft cheese	≤ 45%	60 days	≥ 50%
Asiago fresh and Asiago soft cheese	≤ 32%	1 year	≥ 42%
Blue cheese	≤ 46%	60 days	≥ 50%
Brick cheese	≤ 44%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Caciocavallo siciliano cheese	≤ 40%	90 days @ ≥ 1.7°C	≥ 42%
Cheddar cheese	≤ 39%	60 days @ ≥ 1.7°C (if dairy in ingredients unpasteurised)	≥ 50%
Colby cheese	≤ 40%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Cold-pack and club cheese	NA	60 days @ ≥ 1.7°C (Pasteurised dairy ingredients only)	NA
Cook cheese	≤ 80%	NA	NA
Cottage cheese	≤ 80%	Uncured	NA
Cream cheese	≤ 42%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	NA
Washed curd and soaked curd cheese	≤ 42%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	NA
Edam cheese	≤ 40%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 45%
Gammelost cheese	≤ 52%	Pasteurised	No-fat milk
Gorgonzola cheese	≤ 42%	90 days	≥ 50%
Gouda cheese	≤ 45%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 46%
Granular and stirred curd cheese	≤ 39%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Grated cheese		60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	
Hard grating cheeses	≤ 34%	6 Months	≥ 32%
Gruyere cheese	≤ 39%	90 days	≥ 45%
Hard cheese	≤ 39%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Limburger cheese	≤ 50%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Monterey cheese and Monterey jack cheese	≤ 44%	Pasteurised	≥ 50%
High-moisture jack cheese	≤ 44%	Pasteurised	≥ 50%
Mozzarella cheese and Scamorza cheese	≤ 52%	Pasteurised	≥ 45%
Muenster and Munster cheese	≤ 46%	Pasteurised	≥ 50%
Neufchatel cheese	≤ 65%	Pasteurised	≥ 33%
Nuworld cheese	≤ 46%	60 days	≥ 50%
Parmesan and Reggiano cheese	≤ 32%	10 months	≥ 32%
Provolone cheese	≤ 45%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 45%
Soft ripened cheeses	Un-specified	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Romano cheese	≤ 34%	5 months	≥ 38%
Roquefort cheese	≤ 45%	60 days	≥ 50%
Samsoe cheese	≤ 41%	60 days @ ≥ 1.7°C	≥ 45%
Sap sago cheese	≤ 38%	5 months	Un-specified
Semisoft cheeses	> 39 - ≤ 50%	60 days @ ≥ 1.7°C (if dairy ingredients unpasteurised)	≥ 50%
Swiss and Emmentaler cheese	≤ 41%	60 days	≥ 43%

Appendix 16: References

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